



US009078860B2

(12) **United States Patent**
Szkudlinski et al.

(10) **Patent No.:** **US 9,078,860 B2**
(45) **Date of Patent:** **Jul. 14, 2015**

(54) **VEGF ANALOGS**

(71) Applicant: **Trophogen Inc.**, Rockville, MD (US)

(72) Inventors: **Mariusz W. Szkudlinski**, Rockville, MD (US); **Bruce D. Weintraub**, Rockville, MD (US)

(73) Assignee: **Trophogen, Inc.**, Rockville, MD (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/312,446**

(22) Filed: **Jun. 23, 2014**

(65) **Prior Publication Data**

US 2014/0308264 A1 Oct. 16, 2014

Related U.S. Application Data

(62) Division of application No. 12/089,296, filed as application No. PCT/US2006/039181 on Oct. 6, 2006, now Pat. No. 8,759,285.

(60) Provisional application No. 60/808,106, filed on May 25, 2006, provisional application No. 60/723,917, filed on Oct. 6, 2005.

(51) **Int. Cl.**

A61K 38/18 (2006.01)

A61K 38/16 (2006.01)

A61K 38/17 (2006.01)

C07K 14/475 (2006.01)

C07K 14/52 (2006.01)

A61K 45/06 (2006.01)

A61K 38/00 (2006.01)

(52) **U.S. Cl.**

CPC **A61K 38/1866** (2013.01); **A61K 45/06** (2013.01); **C07K 14/475** (2013.01); **C07K 14/52** (2013.01); **A61K 38/00** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,902,505 A 2/1990 Pardridge et al.
5,008,050 A 4/1991 Cullis et al.
5,017,566 A 5/1991 Bodor
5,380,531 A 1/1995 Chakrabarti et al.
5,604,198 A 2/1997 Poduslo et al.
5,612,034 A 3/1997 Pouletty et al.

6,020,473 A 2/2000 Keyt et al.
6,057,428 A * 5/2000 Keyt et al. 530/399
6,225,449 B1 5/2001 Boime
6,271,436 B1 8/2001 Piedrahita et al.
6,281,408 B1 8/2001 Khillan
6,291,212 B1 9/2001 Sledziewski et al.
6,291,740 B1 9/2001 Bremel et al.
6,361,992 B1 3/2002 Szkudlinski et al.
6,475,796 B1 11/2002 Pollitt et al.
6,485,942 B1 11/2002 Zioncheck et al.
6,750,044 B1 6/2004 Keyt et al.
7,005,505 B1 2/2006 Keyt et al.
2001/0046521 A1 11/2001 Zasloff et al.
2003/0064922 A1 4/2003 Nissen et al.
2004/0265972 A1 12/2004 Weintraub et al.
2005/0054036 A1 3/2005 Bates et al.

FOREIGN PATENT DOCUMENTS

JP 1998-511557 11/1998
JP 2000-507456 6/2000
JP 2001-517075 10/2001
JP 2003-517275 5/2003
WO 90/08832 8/1990
WO 97/08313 3/1997
WO 98/07832 2/1998
WO 00/17360 3/2000
WO 00/25805 5/2000
WO 2005/042575 5/2005
WO 2005/072417 8/2005

OTHER PUBLICATIONS

Keyt et al., "Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptor-selective VEGF variants by site-directed mutagenesis," *Journal of Biological Chemistry*, 271 (10):5638-5646 (1998).
Siemeister et al., "An antagonistic vascular endothelial growth factor (VEGF) variant inhibits VEGF-stimulated receptor autophosphorylation and proliferation of human endothelial cells," *Proc. Natl Acad Sci USA*, 95(8): 4625-4629 (1998).
Li et al. "Receptor-selective Variants of Human Vascular Endothelial Growth Factor," *Journal of Biological Chemistry*, 275 (38): 29823-8 (1998).

* cited by examiner

Primary Examiner — Christine J Saoud

Assistant Examiner — Jon M Lockard

(74) *Attorney, Agent, or Firm* — Morgan Lewis & Bockius LLP

(57) **ABSTRACT**

Modified VEGF proteins that inhibit VEGF-mediated activation or proliferation of endothelial cells are disclosed. The analogs may be used to inhibit VEGF-mediated activation of endothelial cells in angiogenesis-associated diseases such as cancer, inflammatory diseases, eye diseases, and skin disorders.

22 Claims, 6 Drawing Sheets

Figure 1A

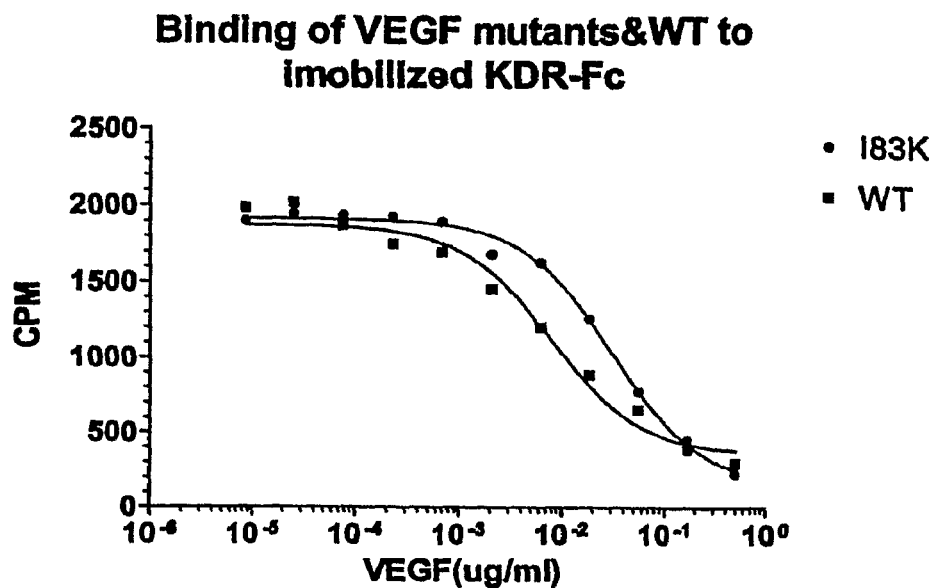


Figure 1B

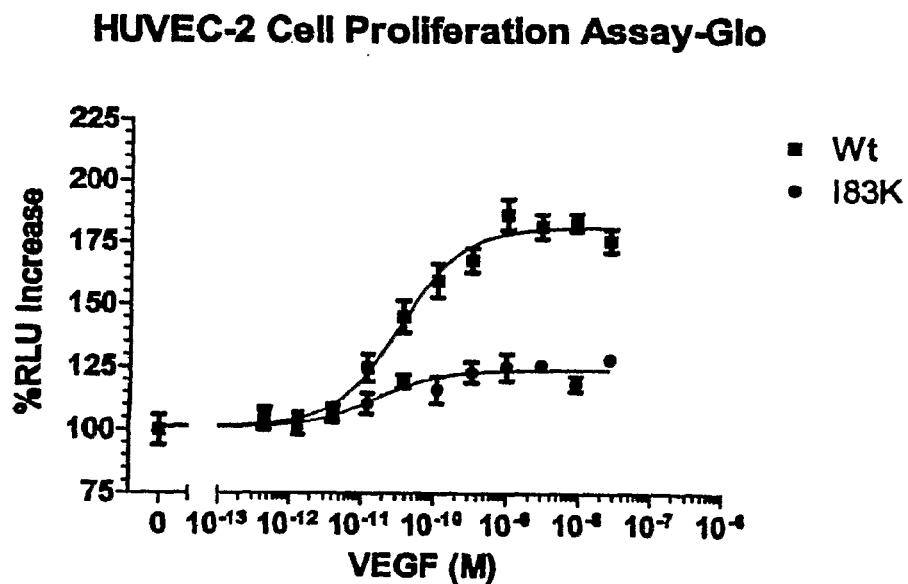


Figure 2A

Yeast VEGF Mutants Binding Assay

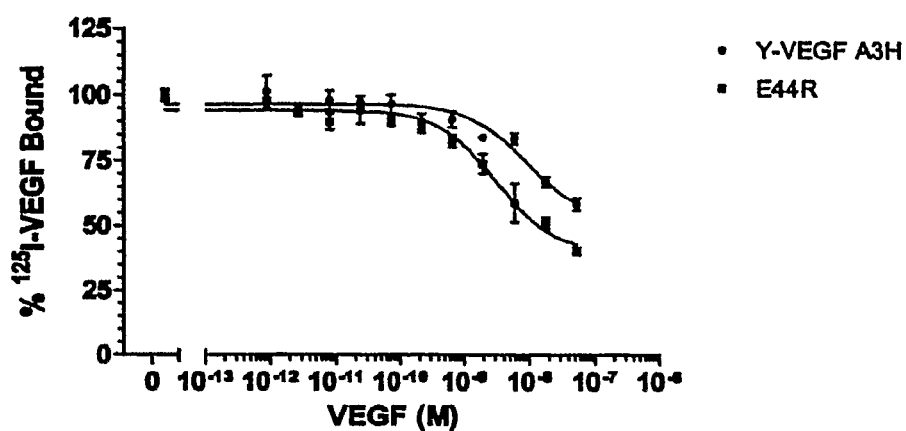


Figure 2B

HUVEC-2 Cell Proliferation Assay-Glo

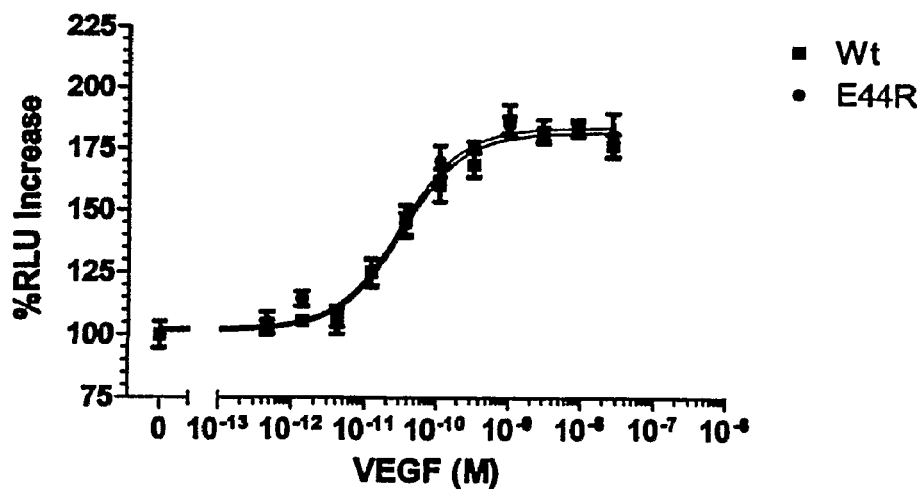


Figure 3A

Yeast VEGF Mutants Binding Assay

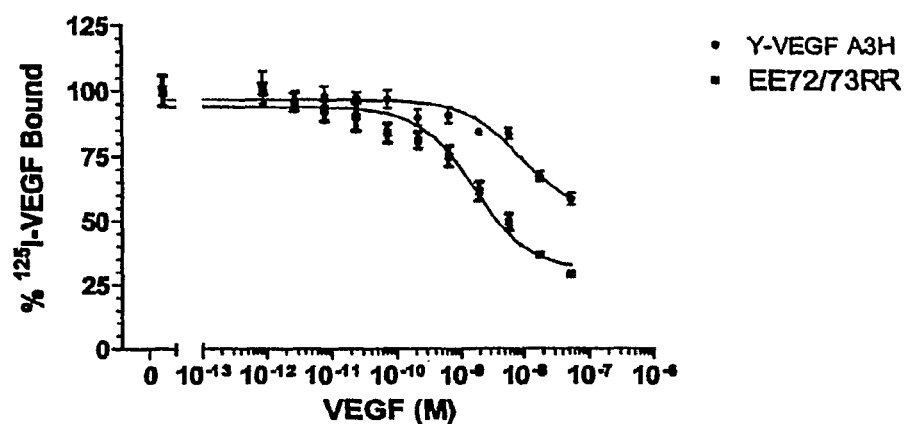


Figure 3B

HUVEC-2 Cell Proliferation Assay-Glo

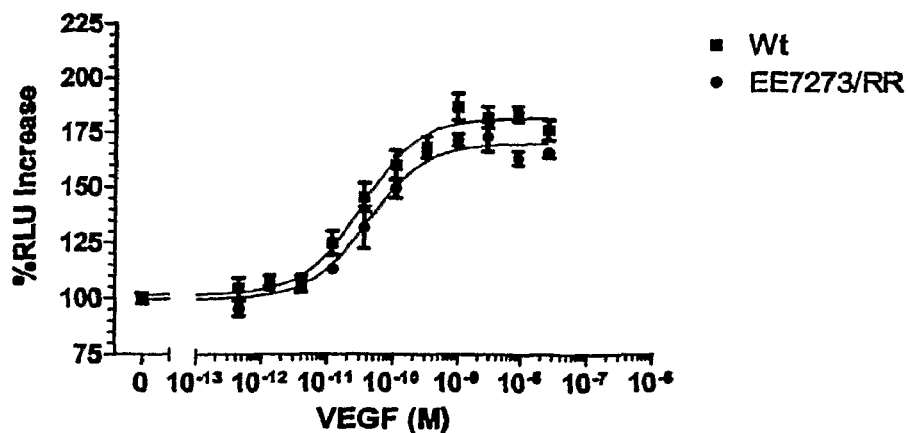


Figure 4

VEGF Competition Binding on KDR-Fc E44R and EE72/73RR

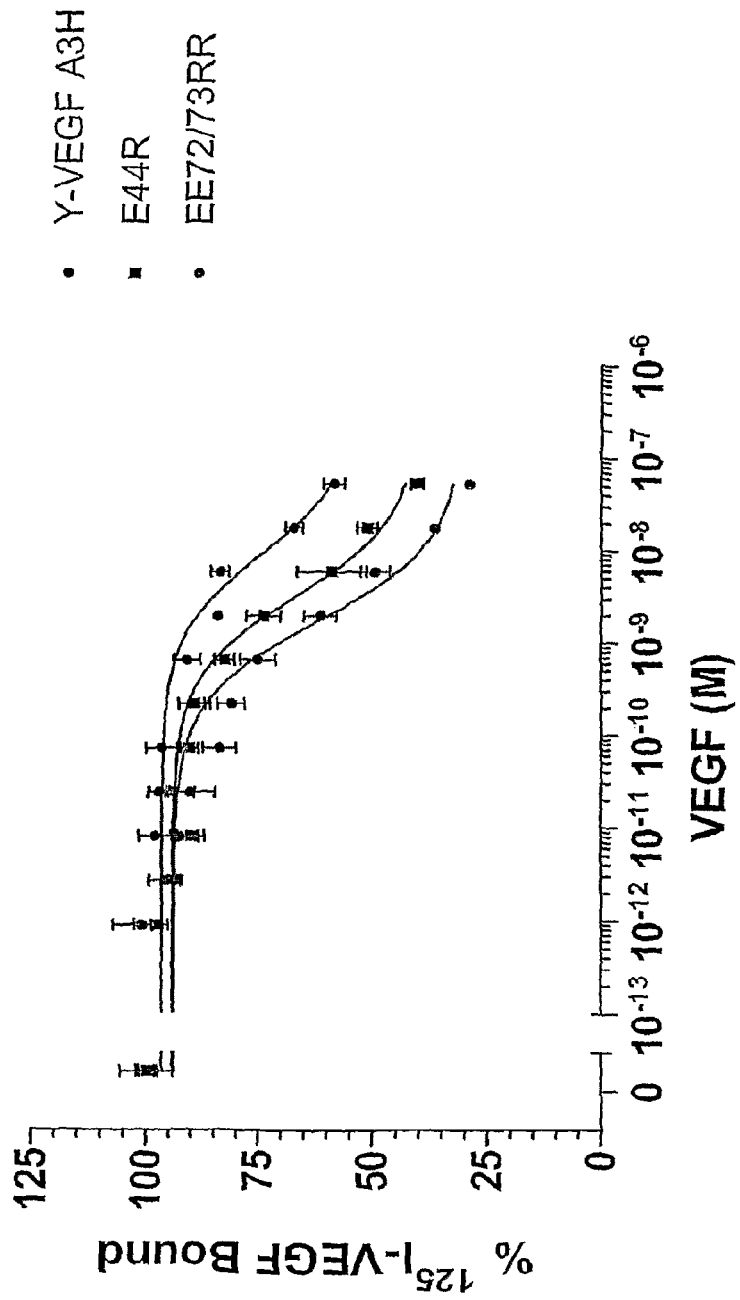


Figure 5

Yeast construct KDR Binding Assay

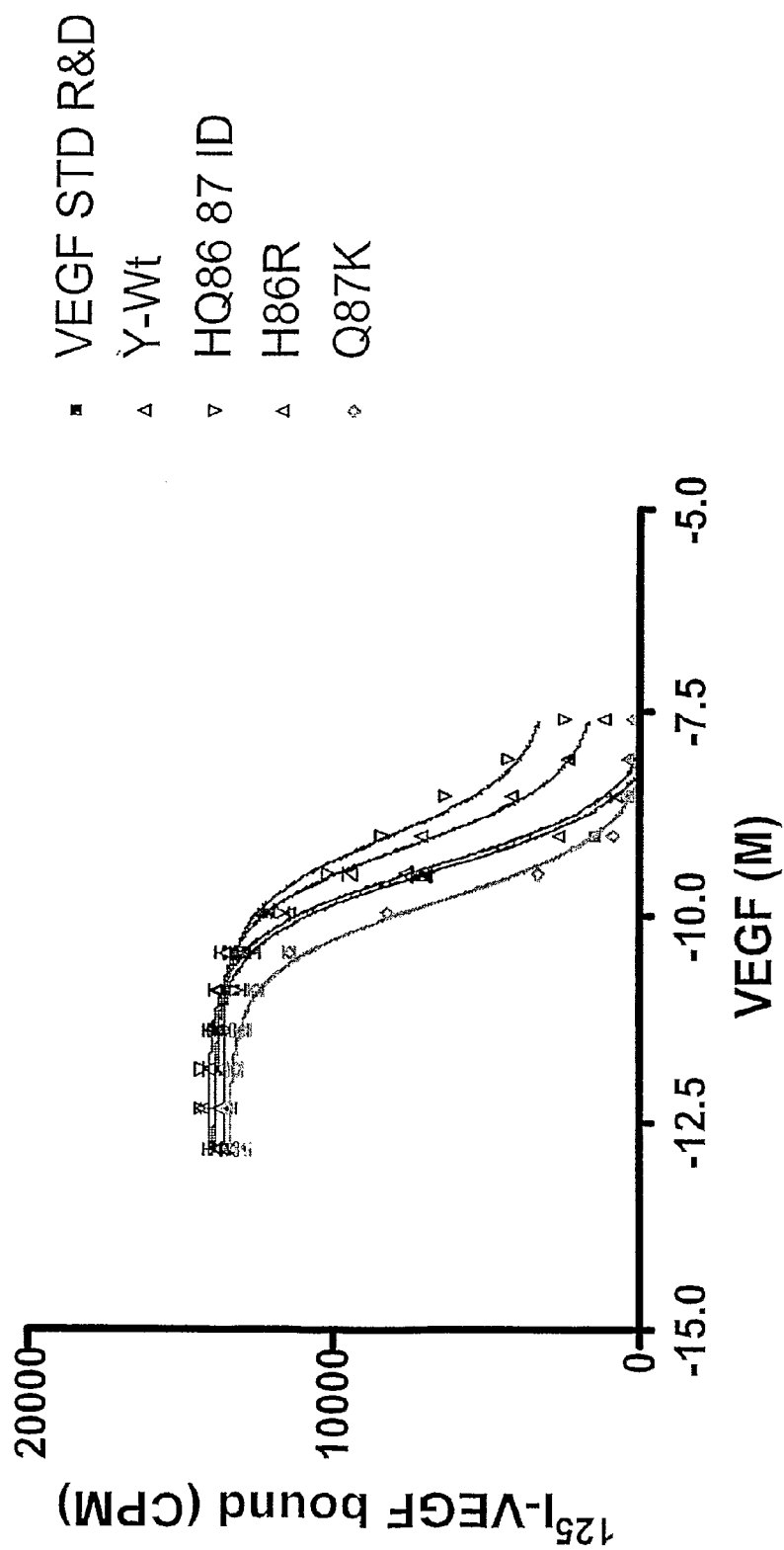
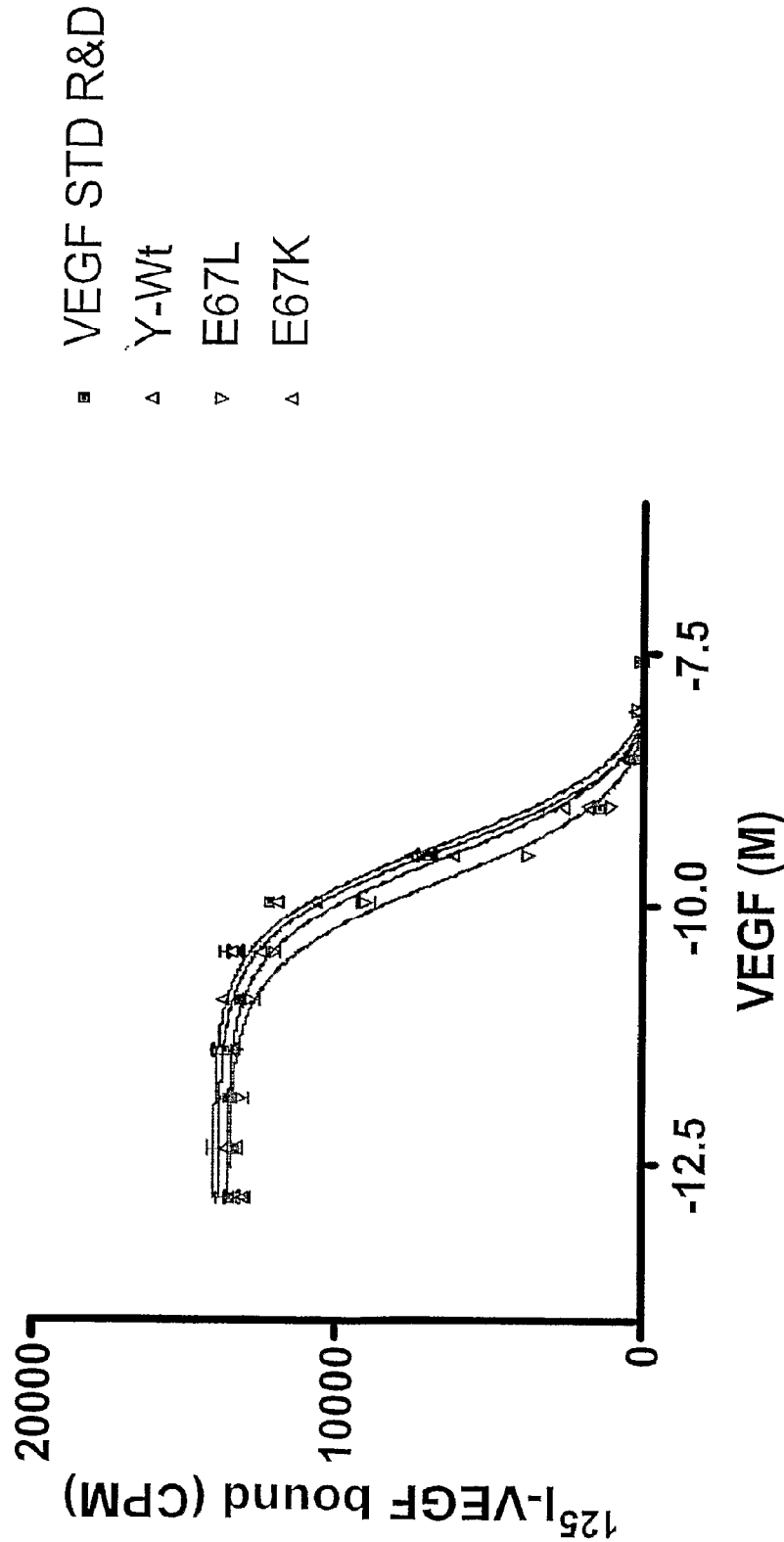


Figure 6

Yeast construct KDR Binding Assay



1

VEGF ANALOGS**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 60/723,917, filed Oct. 6, 2005, and U.S. Provisional Application No. 60/808,106, filed May 25, 2006, which are herein incorporated by reference in their entireties.

SEQUENCE LISTING SUBMISSION VIA EFS-WEB

A computer readable text file, entitled "056815-5005-01-SequenceListing.txt" created on or about Jun. 20, 2014, with a file size of about 146 kb contains the sequence listing for this application and is hereby incorporated by reference in its entirety.

FIELD OF INVENTION

This application relates to the design and use of vascular endothelial growth factor (VEGF) analogs as VEGF receptor antagonists to inhibit or reduce angiogenesis for the treatment of conditions and diseases associated with angiogenesis. The application also discloses VEGF analogs that exhibit increased receptor binding affinity to native receptors such as KDR.

BACKGROUND OF INVENTION

Vascular endothelial growth factors (VEGFs) regulate blood and lymphatic vessel development. They are predominantly produced by endothelial, hematopoietic and stromal cells in response to hypoxia and stimulation with growth factors such as transforming growth factors, interleukins and platelet-derived growth factor.

In mammals, VEGFs are encoded by a family of genes and include VEGF-A, VEGF-B, VEGF-C, VEGF-D and Placenta like Growth Factor (PLGF). Highly related proteins include orf virus-encoded VEGF-like proteins referred to as VEGF-E and a series of snake venoms referred to as VEGF-F. VEGFs and VEGF-related proteins are members of the Platelet Derived Growth Factor (PDGF) supergene family of cystine knot growth factors. All members of the PDGF supergene family share a high degree of structural homology with PDGF (see U.S. patent application Ser. No. 09/813,398 which is herein incorporated by reference in its entirety).

VEGF-A, VEGF-B and PLGF are predominantly required for blood vessel formation, whereas VEGF-C and VEGF-D are essential for the formation of lymphatic vessels. Angiogenesis is the process by which new blood vessels or lymphatic vessels form by developing from pre-existing vessels. The process is initiated when VEGFs bind to receptors on endothelial cells, signaling activation of endothelial cells. Activated endothelial cells produce enzymes which dissolve tiny holes in the basement membrane surrounding existing vessels. Endothelial cells then begin to proliferate and migrate out through the dissolved holes of the existing vessel to form new vascular tubes (Alberts et al., 1994, *Molecular Biology of the Cell*. Garland Publishing, Inc., New York, N.Y. 1294 pp.).

Three type III receptor tyrosine kinases are activated by VEGFs during angiogenesis: fms-like tyrosine kinase (Flt-1, also known as VEGFR1), kinase domain receptor or kinase insert domain-containing receptor (KDR, also known as VEGFR2 and Flk-1) and Flt-4 (also known as VEGFR3).

2

KDR is the predominant receptor in angiogenic signaling, whereas Flt-1 is associated with the regulation of blood vessel morphogenesis and Flt-4 regulates lymphangiogenesis. These receptors are expressed almost exclusively on endothelial cells, with a few exceptions such as the expression of Flt-1 in monocytes where it mediates chemotaxis (Barleon et al., 1996, *Blood*. 87: 3336-3343).

VEGF receptors are closely related to Fms, Kit and PDGF receptors. They consist of seven extracellular immunoglobulin (Ig)-like domains, a transmembrane (TM) domain, a regulatory juxtamembrane domain, an intracellular tyrosine kinase domain interrupted by a short peptide, the kinase insert domain, followed by a sequence carrying several tyrosine residues involved in recruiting downstream signaling molecules. Mutation analysis of the extracellular domains of Flt-1 and KDR show that the second and third Ig-like domains constitute the high-affinity ligand-binding domain for VEGF with the first and fourth Ig domains apparently regulating ligand binding and receptor dimerization, respectively (Davis-Smyth et al., 1998, *J. Biol. Chem.* 273: 3216-3222; Fuh et al., 1998, *J. Biol. Chem.* 273: 11197-11204; and Shin-kai et al., 1998, *J. Biol. Chem.* 273: 31283-31288). Receptor tyrosine kinases are activated upon ligand-mediated receptor dimerization (Hubbard, 1991, *Prog. Biophys. Mol. Biol.* 71: 343-358; Jiang and Hunter, 1999, *Curr. Biol.* 9: R568-R571; and Lemmon and Schlessinger, 1998, *Methods Mol. Biol.* 84: 49-71). Signal specificity of VEGF receptors is further modulated upon recruitment of coreceptors, such as neuropilins, heparin sulfate, integrins or cadherins.

VEGF molecules interact with one or more tyrosine kinase receptors during angiogenesis. For instance, VEGF-A acts predominantly through KDR and Flt-1. VEGF-C and VEGF-D similarly are specific ligands for KDR and VEGFR3. PLGF and VEGF-B are believed to bind only to Flt-1. Viral VEGF-E variants activate KDR. VEGF-F variants interact with either VEGFR3 or KDR.

In addition to the two classical receptors, there are several membrane or soluble receptors modulating VEGF bioactivity and angiogenesis. For instance, neuropilin-1 and neuropilin-2 interact with both KDR and Flt-1, respectively, stimulating signaling of those receptors. Isoforms of VEGF-A, VEGF-B, PLGF-2 have been shown to bind to neuropilin-1 (Soker et al., 1998, *Cell*. 92: 735-745; Makinen et al., 1999, *J. Biol. Chem.* 274: 21217-21222; and Migdal et al., 1998, *J. Biol. Chem.* 273: 22272-22278). VEGF isoforms capable of interacting with neuropilin, i.e., those isoforms with exon 7 or 6 and 7, are also capable of interacting with heparin sulfate.

Although VEGF-A is the best characterized of the VEGF proteins, the molecular basis of the interaction between VEGF-A and KDR and Flt-1 is not well understood. Although VEGFR1 binds VEGF-A with a 50-fold higher affinity than KDR, KDR is considered to be the major transducer of VEGF-A angiogenic effects, i.e., mitogenicity, chemotaxis and induction of tube formation (Binetruy-Tourniere et al., *supra*). There is, however, growing evidence that Flt-1 has a significant role in hematopoiesis and in the recruitment of monocytes and other bone-marrow derived cells that may home in on tumor vasculature and promote angiogenesis (Hattori et al., 2002, *Nature Med.* 8: 841-849; Gerber et al., 2002, *Nature*. 417: 954-958; and Luttun et al., 2002, *Nature Med.* 8: 831-840). Further, in some cases Flt-1 is expressed by tumor cells and may mediate a chemotactic signal, thus potentially extending the role of this receptor in cancer growth (Wey et al., 2005, *Cancer*. 104: 427-438).

A single VEGF-A homodimer induces dimerization of two KDR receptors and autophosphorylation of their cytoplasmic portions. Previous studies suggested that by analogy to

glycoprotein hormones, the charged amino acid residues in the peripheral loops of VEGF-A are also critical in providing high affinity electrostatic interactions with its respective receptors (Szkudlinski et al., 1996, *Nat. Biotechnol.* 14(10): 1257-63; Fuh et al., *supra*; Muller et al., 1997, *Proc. Natl. Acad. Sci. U.S.A.* 94(14): 7192-7; Keyt et al., 1996, *J. Biol. Chem.* 271(10): 5638-46). However, it should be noted that many mutations in VEGF-A have no major effect on receptor binding affinity. Mutations in the peripheral loops of VEGF primarily have resulted in loss-of-function. Further, there appear to be no previous amino acid substitutions increasing binding affinity to KDR more than 2-fold.

Angiogenesis is responsible for beneficial biological events such as wound healing, myocardial infarction repair, and ovulation. On the other hand, angiogenesis is also responsible for causing or contributing to diseases such as growth and metastasis of solid tumors (Isayeva et al., 2004, *Int. J. Oncol.* 25(2):335-43; Takeda et al., 2002, *Ann Surg. Oncol.* 9(7):610-16); atherosclerosis; abnormal neovascularization of the eye as seen in diseases such as retinopathy of prematurity, diabetic retinopathy, retinal vein occlusion, and age-related macular degeneration (Yoshida et al., 1999, *Histol Histopathol.* 14(4):1287-94; Aiello, 1997, *Ophthalmic Res.* 29(5):354-62); chronic inflammatory conditions such as rheumatoid arthritis osteoarthritis, and septic arthritis; neurodegenerative disease (Ferrara, N., 2004, *Endocr. Rev.* 25: 581-611); placental insufficiency, i.e., preeclampsia (Ferrara, *supra*); and skin diseases such as dermatitis, psoriasis, warts, cutaneous malignancy, decubitus ulcers, stasis ulcers, pyogenic granulomas, hemangiomas, Kaposi's sarcoma, hypertrophic scars, and keloids (Arbiser, 1996, *J. Am. Acad. Dermatol.* 34(3):486-97). During rheumatoid arthritis, for example, endothelial cells become activated and express adhesion molecules and chemokines, leading to leukocyte migration from the blood into the tissue. Endothelial cell permeability increases, leading to edema formation and swelling of the joints (Middleton et al., 2004, *Arthritis Res. Ther.* 6(2):60-72).

VEGF, in particular VEGF-A, has been implicated in many of the diseases and conditions associated with increased, decreased, and/or dysregulated angiogenesis (Binetruy-Tourniere et al., 2000, *EMBO J.* 19(7): 1525-33). For instance, VEGF has been implicated in promoting solid tumor growth and metastasis by stimulating tumor-associated angiogenesis (Lu et al., 2003, *J. Biol. Chem.* 278(44): 43496-43507). VEGF is also a significant mediator of intraocular neovascularization and permeability. Overexpression of VEGF in transgenic mice results in clinical intraretinal and subretinal neovascularization, and the formation of leaky intraocular blood vessels detectable by angiography, as seen in human eye disease (Miller, 1997, *Am. J. Pathol.* 151(1): 13-23). Additionally, VEGF has been identified in the peritoneal fluid of women with unexplained infertility and endometriosis (Miedzybrodzki et al., 2001, *Ginek. Pol.* 72(5): 427-430), and the overexpression of VEGF in testis and epididymis has been found to cause infertility in transgenic mice (Korpelainen et al., 1998, *J. Cell Biol.* 143(6): 1705-1712). Recently, VEGF-A has been identified in the synovial fluid and serum of patients with rheumatoid arthritis (RA), and its expression is correlated with disease severity (Clavel et al., 2003, *Joint Bone Spine.* 70(5): 321-6). Given the involvement of pathogenic angiogenesis in such a wide variety of disorders and diseases, inhibition of angiogenesis, and particularly of VEGF signaling, is a desirable therapeutic goal.

Inhibition of angiogenesis and tumor inhibition has been achieved by using agents that either interrupt VEGF-A and

KDR interaction and/or block the KDR signal transduction pathway including: peptides that block binding of VEGF to KDR (Binetruy-Tourniere et al., 2000, *EMBO J.* 19(7): 1525-33); antibodies to VEGF (Kim et al., 1993, *Nature* 362, 841-844; Lanai et al., 1998, *J. Cancer* 77, 933-936; Margolin et al., 2001, *J. Clin. Oncol.* 19, 851-856); antibodies to KDR (Lu et al., 2003, *supra*; Zhu et al., 1998, *Cancer Res.* 58, 3209-3214; Zhu et al. 2003, *Leukemia* 17, 604-611; Prewett et al., 1999, *Cancer Res.* 59, 5209-5218); soluble receptors (Holash et al., 2002, *Proc. Natl. Acad. Sci. USA* 99, 11393-11398; Clavel et al. *supra*); tyrosine kinase inhibitors (Fong et al., 1999, *Cancer Res.* 59, 99-106; Wood et al., 2000, *Cancer Res.* 60, 2178-2189; Grosios et al., 2004, *Inflamm Res.* 53(4):133-42); anti-VEGF immunotoxins (Olson et al., 1997, *Int. J. Cancer* 73, 865-870); ribozymes (Pavco et al., 2000, *Clin. Cancer Res.* 6, 2094-2103); antisense mediated VEGF suppression (Forster et al., 2004, *Cancer Lett.* 20: 212(1):95-103); RNA interference (Takei et al., 2004, *Cancer Res.* 64(10):3365-70; Reich et al., 2003, *Mol. Vis.* 9:210-6); and undersulfated, low molecular weight glycol-split heparin (Pisano et al., 2005, *Glycobiology.* 15(2) 1-6). Some of these treatments, however, have resulted in undesirable side effects. For instance, Genentech's Avastin, a monoclonal antibody that targets VEGF, has been reported to cause an increase in serious arterial thromboembolic events in some colon cancer patients and serious, and in some cases even fatal, hemoptysis in non-small cell lung cancer patients (Ratner, 2004, *Nature Biotechnol.* 22(10):1198). More recently, Genentech has reported that gastrointestinal perforations were observed in 11% of ovarian cancer patients (5 women out of 44 in trial) treated with Avastin (Genentech Press Release dated Sep. 23, 2005). Similarly, the first VEGF-targeting drug, Pfizer's receptor tyrosine kinase inhibitor SU5416, exhibited severe toxicities that included thromboembolic events, prompting Pfizer to discontinue development (Ratner, *supra*). Given the wide variety of patients that stand to benefit from the development of effective anti-angiogenic treatments and the drawbacks of some known anti-angiogenesis treatments, there remains a need for novel anti-angiogenic therapeutics.

SUMMARY OF INVENTION

This invention encompasses VEGF analogs and nucleic acids encoding the same, which exhibit strong binding affinity for one or more native VEGF receptors compared to wild-type VEGF. The invention also encompasses VEGF analogs and nucleic acids encoding same, which exhibit a dissociation of receptor binding affinity and bioactivity. Specifically, the in vivo and in vitro bioactivities of the disclosed analogs are substantially decreased compared to wild-type VEGF, whereas the binding affinity to one or more native receptors is about the same or substantially increased compared to wild-type VEGF. The VEGF analogs may demonstrate at least about a three to four fold increase in receptor binding affinity to a native receptor such as KDR.

In one embodiment of the invention, the VEGF analogs are modified VEGF homodimers or heterodimers. These molecules contain at least one mutation which can be present in one or both subunits of the VEGF molecule. In one embodiment of the invention, the VEGF analog containing the one or more mutations is VEGF-A. The VEGF-A analog can be any VEGF-A isoform, for instance, an isoform of 121, 145, 148, 165, 183, 189, or 206 amino acids. In one embodiment, the VEGF-A analog of the invention is a VEGF_{165b} isoform. In another embodiment, the VEGF molecule containing one or more mutations is VEGF-B, VEGF-C, VEGF-D or PlGF.

5

The present invention includes a VEGF fusion protein containing one or more mutations in one or more subunits. The VEGF fusion protein of the invention includes at least one VEGF subunit, i.e., subunit, fused to at least one subunit of a different protein, including, but not limited to, other cystine knot growth factors or glycoproteins. For instance, the invention includes a chimera VEGF analog in which the VEGF molecule contains a VEGF-A subunit fused to a VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, PDGF or PIGF subunit; a VEGF-B subunit fused to a VEGF-A, VEGF-C, VEGF-D, VEGF-E, VEGF-F, PDGF or PIGF subunit; a VEGF-C subunit fused to a VEGF-A, VEGF-B, VEGF-D, VEGF-E, VEGF-F, PDGF or PIGF subunit; a VEGF-D subunit fused to a VEGF-A, VEGF-B, VEGF-C, VEGF-E, VEGF-F, PDGF or PIGF subunit; or a PIGF subunit fused to a VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, or PDGF subunit. The subunits may optionally be separated by a linker peptide. The invention also includes different isoforms of the same VEGF fused together, e.g., VEGF₁₆₅ subunit fused to VEGF_{165b}.

In one embodiment, the VEGF analog is a single chain molecule. For instance, the VEGF analog of the invention includes two VEGF subunits, i.e., monomers, linked together via a linker peptide. One or both linked subunits can contain one or more basic amino acid substitutions. Further, the linked subunits can be different VEGF protein subunits and can be different isoforms of the same subunit. For instance, the present invention includes a wild-type VEGF₁₆₅ subunit linked via a GS linker to a VEGF₁₆₅ subunit with a I83K amino acid substitution.

In another embodiment of the invention, a VEGF-A, VEGF-B, VEGF-C, VEGF-D, or PIGF subunit or dimer comprising one or more mutations is fused to a toxin. The peptide of this embodiment can be useful for the targeting and destruction of tumor cells.

The VEGF analogs of the invention include one or more basic amino acid substitutions, such as lysine or arginine, from the group of positions 44, 67, 72, 73, 83, and 87. In one embodiment of the invention, the VEGF analog contains a basic amino acid substitution at position 83 and optionally one or more basic amino acid substitutions at positions 44, 67, 72 and 73. For instance, the invention includes a VEGF analog with a I83K mutation. The invention also includes, for instance, a VEGF analog with basic amino acids at positions 72, 73 and 83.

VEGF analogs with the basic amino acid substitutions described herein may contain additional amino acid substitutions to further increase receptor binding affinity to KDR and/or decrease receptor binding affinity to neuropilin-1. For instance, the invention includes mutations at positions 146 and 160 in the which act to disrupt the neuropilin-1 binding site.

Analog of the invention can also contain additional amino acid substitutions which confer enhanced stability and increased serum half-life. For instance, the invention includes amino acids substitutions which eliminate proteolytic cleavage sites such substitutions at positions 111 and 148.

The VEGF receptor antagonists of the present invention can exhibit increased plasma half-life as compared to wild-type VEGF. This may be accomplished by further modifying a VEGF analog by methods known in the art to increase half-life or, alternatively, increased plasma half-life may be an inherent characteristic of a VEGF analog. The VEGF receptor antagonists of the invention can also exhibit an increase in rate of absorption and/or decreased duration of action compared to wild-type VEGF.

6

The modified analogs of the invention act as VEGF receptor antagonists and thus provide a long awaited solution for patients suffering from a wide spectrum of diseases and conditions associated with angiogenesis. The VEGF receptor antagonists can be administered to a patient alone or in conjunction with another VEGF receptor antagonist, an anti-cancer drug, or an anti-angiogenesis drug for the treatment of disease associated with angiogenesis, including but not limited to, solid tumor cancers, hemangiomas, rheumatoid arthritis, osteoarthritis, septic arthritis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, vascular restenosis, arteriovenous malformations, meningiomas, neovascular glaucoma, psoriasis, Kaposi's Syndrome, angiofibroma, hemophilic joints, hypertrophic scars, Osler-Weber syndrome, pyogenic granuloma, retrolental fibroplasias, scleroderma, trachoma, von Hippel-Lindau disease, vascular adhesion pathologies, synovitis, dermatitis, neurological degenerative diseases, preeclampsia, unexplained female infertility, endometriosis, unexplained male infertility, pterygium, wounds, sores, skin ulcers, gastric ulcers, and duodenal ulcers.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a graph comparing binding of the I83K mutant and wild-type VEGF-A to KDR. FIG. 1B is a graph showing a decrease in proliferation of HUVEC-2 endothelial cells in the presence of the I83K VEGF-A mutant compared to wild-type VEGF-A.

FIG. 2A is a graph comparing binding of the E44R analog and wild-type VEGF-A to KDR. FIG. 2B is a graph comparing HUVEC-2 cell proliferation in the presence of the E44R VEGF-A analog versus wild-type VEGF-A.

FIG. 3A is a graph comparing binding of the E72R+E73R VEGF mutant and wild-type VEGF-A to KDR. FIG. 3B is a graph comparing HUVEC-2 cell proliferation in the presence of the E72R+E73R VEGF mutant versus wild-type VEGF-A.

FIG. 4 is a graph comparing binding of E44R and E72/73R mutants to wild-type VEGF-A.

FIG. 5 is a graph comparing binding of Q87K mutant to wild-type VEGF-A.

FIG. 6 is a graph comparing binding of E67K mutant to wild-type VEGF-A.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides modified angiogenic growth factors of the vascular endothelial growth factor (VEGF) family which exhibit surprising activity as VEGF receptor antagonists. As VEGF receptor antagonists, the compounds of the invention have "anti-angiogenic" properties. Being "modified" means that, while the protein contains an amino acid sequence which differs from a wild-type VEGF of interest, i.e., human VEGF or animal VEGF, the sequence has not been changed such that it is identical to the known VEGF sequence of other species. The terms "mutated" and "substituted" are used interchangeably herein to refer to modified amino acid residues. The terms "modified VEGF molecules", "modified VEGF proteins", "VEGF analogs", "VEGF receptor antagonists", "VEGF chimeras", "VEGF fusion proteins" and "VEGF single chain molecules" are used interchangeably herein to refer to modified VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF analog molecules.

"Antagonists" are used interchangeably herein to refer to molecules which act to block, inhibit or reduce the natural, biological activities of VEGF, such as the induction of angiogenesis. The term "anti-angiogenic" as used herein means

that the modified VEGF molecules of the invention block, inhibit or reduce the process of angiogenesis, or the process by which new blood or lymphatic vessels form from pre-existing vessels. The activities of the VEGF analogs of the invention disrupt normal VEGF/receptor signaling which usually occurs when VEGF binds to a receptor. Accordingly, the analogs of the invention are VEGF receptor antagonists. Without wishing to be bound by a theory, it is believed that the VEGF analogs of the invention disrupt the dimerization of KDR necessary for signaling.

Inhibition of angiogenesis may be complete or partial. The VEGF receptor antagonist may inhibit angiogenesis at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, and at least about 100% in vitro and in vivo. Inhibition of angiogenesis can be measured by a skilled artisan by methods known in the art. The determination of inhibition of angiogenesis can include the use of negative and/or positive controls. For instance, a skilled artisan can conclude that a VEGF analog of the invention inhibits VEGF-induced angiogenesis by comparing angiogenesis in a subject treated with a VEGF analog of the invention to a similar subject not treated with a VEGF analog.

The modified VEGF molecules of the invention display increased receptor binding affinity or similar receptor binding affinity to one or more native VEGF receptors compared to that of wild-type VEGF. As used herein, a native VEGF receptor is an unmodified receptor that specifically interacts with VEGF. For instance, an endogenous VEGF receptor is a native VEGF receptor. In one embodiment of the invention, the native receptor is KDR. KDR is a receptor of VEGF-A, VEGF-C, VEGF-D, VEGF-E and VEGF-F. In another embodiment, the native receptor is Flt-1. Flt-1 is a receptor of VEGF-A, VEGF-B and PlGF.

"Receptor binding affinity" refers to the ability of a ligand to bind to a receptor in vivo or in vitro and can be assessed by methods readily available in the art including, but not limited to, competitive binding assays and direct binding assays. As used herein, receptor binding affinity refers to the ability of VEGF molecules to bind to native VEGF receptors, including, but not limited to, Flt-1 (also known as VEGF-R1), KDR (also known as VEGF-R2) and Flt-4 (also known as VEGF-R3). For instance, the modified VEGF-A molecules of the invention display increased binding receptor affinity or similar binding affinity to KDR compared to wild-type VEGF-A. In one embodiment, the increase in receptor binding affinity of the modified VEGF molecules of the invention is at least about 1.25 fold, at least about a 1.5 fold, at least about a 1.75 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold or at least about 10 fold greater than that of wild-type VEGF.

In another embodiment, the modified VEGF exhibits a receptor binding affinity to KDR and/or other receptor that is involved in angiogenesis that is similar or comparable to that of wild-type VEGF. Similar or comparable receptor binding affinity is at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 97% or more of that of wild-type VEGF. For instance, the invention includes VEGF-A analogs exhibiting about 75% to 85%, about 85% to 95% and about 95% to 100% the receptor binding affinity exhibited by wild-type VEGF.

The present invention also includes VEGF analogs which display increased or similar receptor binding affinity to at least one native receptor but display decreased receptor binding affinity to another native receptor. For instance, VEGF-A

analog of the invention may display increased or similar receptor binding affinity to KDR compared to wild-type VEGF-A, but may display decreased receptor binding affinity to Flt-1, neuropilin-1 or neuropilin-2 compared to wild-type VEGF-A.

The VEGF analogs of the invention also display a decrease in bioactivity compared to wild-type VEGF. "Bioactivity" refers to the natural, biological activities of VEGF in vivo and in vitro, including, but not limited to, the ability of VEGF to induce cell proliferation in endothelial cells. A decrease in bioactivity results in a decrease in angiogenesis. In one embodiment of the invention, the VEGF analogs of the invention display a decrease in bioactivity compared to wild-type VEGF of the same isoform. For instance, a VEGF₁₆₅ analog of the invention can display a decrease in bioactivity compared to wild-type VEGF₁₆₅, or a VEGF_{165b} analog can display a decrease in bioactivity compared to wild-type VEGF_{165b}.

Bioactivity can be assessed by several methods known in the art, including, but not limited to, in vitro cell viability assays which assay the viability of endothelial cells such as human umbilical vein endothelial cells (HUVEC) upon exposure to VEGF. A decrease in endothelial cell viability of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% or more compared to resulting from exposure to wild-type VEGF is indicative of a decrease in bioactivity.

Bioactivity can be assessed in vivo as well. For instance, bioactivity can be assessed in vivo in a subject with a tumor by detecting a lack of increase in angiogenesis around a tumor. The detection of a lack of increase in angiogenesis can be accomplished by several methods known in the art including, but not limited to, an in vivo matrigel migration assay, a disc angiogenesis assay, an assay comprising a dorsal skinfold chamber in mice, a corneal transplant and a sponge implant model of angiogenesis. In one embodiment, angiogenesis is assessed by comparing angiogenesis of and around the tumor to that of a tumor of similar type, size and location in an untreated subject. Biopsy methods as known in the art can be used to extract tissue and analyze for vessel formation.

"Dissociation" of receptor binding affinity and bioactivity refers to the concept that receptor binding affinity and bioactivity are not correlated. In comparison, receptor binding affinity and bioactivity are correlated for wild-type VEGF proteins such as wild-type VEGF-A. An increase in receptor binding ability, for example, would be expected to result in an increase in bioactivity for wild-type VEGF-A. On the other hand, the modified VEGF molecules of the invention demonstrate a similar receptor binding affinity or an increase in receptor binding affinity as compared to wild-type VEGF but a decrease in bioactivity as compared to wild-type VEGF.

Mammalian VEGFs are produced in multiple isoforms due to alternative splicing of a family of related genes. The present invention describes VEGF analogs which correspond to VEGF isoforms involved in angiogenesis. The VEGF analogs of the present invention can be created using any VEGF isoform unless otherwise indicated.

VEGF-A can exist in isoforms including, but not limited to, 121, 145, 148, 165, 183, 189, and 206 amino acids, respectively. The three main mRNA species are VEGF₁₂₁, VEGF₁₆₅ and VEGF₁₈₉. As used herein, VEGF₁₂₁ (SEQ ID NO.: 6), VEGF₁₄₅ (SEQ ID NO.: 8), VEGF₁₄₈ (SEQ ID NO.: 10), VEGF₁₆₅ (SEQ ID NO.: 4), VEGF_{165b} (SEQ ID NO.: 13), VEGF₁₈₃ (SEQ ID NO.: 15), VEGF₁₈₉ (SEQ ID NO.: 17) and

VEGF₂₀₆ (SEQ ID NO.: 19) are isoforms of VEGF-A capable of being modified to possess anti-angiogenic properties. The amino acid positions described herein are based on a VEGF molecule lacking a leader sequence such as the leader sequence of SEQ ID NO.: 3. The amino acid sequences of VEGF-A isoforms with leader sequence are the sequences of SEQ ID NOs.: 2, 5, 7, 9, 12, 14, 16 and 18.

The various isoforms of VEGF-A share a common amino-terminal domain consisting of 110 amino acids. VEGF-A isoforms have a receptor binding domain encoded by exons 2-5. The most notable difference between the isoforms are found in the neuropilin and heparin binding domains which are encoded by exons 6a, 6b, 7a and 7b.

The most common VEGF-A isoform is VEGF₁₆₅. The nucleic acid encoding VEGF₁₆₅ is the sequence of SEQ ID NO.: 1. Recently, an endogenous splice variant referred to as VEGF_{165b} was described which contains sequences encoded by exon 9, instead of exon 8, at the carboxy terminus. The nucleic acid molecule encoding this protein is the sequence of SEQ ID NO.: 11. VEGF_{165b} (SEQ ID NO.: 12 with leader sequence; SEQ ID NO.: 13 without leader sequence) inhibited VEGF signaling in endothelial cells when added with VEGF₁₆₅ (see Woolard et al., 2004, Cancer Research. 64: 7822-7835; see also U.S. 2005/0054036 which is herein incorporated by reference in its entirety).

In one embodiment of the invention, the VEGF analogs are VEGF-A analogs. VEGF-A analogs include "modified VEGF-A proteins", "VEGF-A receptor antagonists", "VEGF-A chimeras", "VEGF-A fusion proteins" and "VEGF-A single chain molecules." A VEGF-A analog is a VEGF-A molecule containing at least one modified VEGF-A subunit.

VEGF-B exists in two isoforms, VEGF-B₁₆₇ (SEQ ID NO.: 48) and VEGF-B₁₈₆ (SEQ ID NO.: 50) (Makinen et al., 1999, 3. Biol. Chem. 274: 21217-21222). In one embodiment of the invention, the VEGF analog is a VEGF-B analog. VEGF-B analogs include "modified VEGF-B proteins", "VEGF-B analogs", "VEGF-B receptor antagonists", "VEGF-B chimeras", "VEGF-B fusion proteins" and "VEGF-B single chain molecules." A VEGF-B analog is a VEGF-B molecule containing at least one modified VEGF-B subunit.

VEGF-C is produced as a propeptide (SEQ ID NO.: 51) that is proteolytically cleaved to form a 21-kd active protein (Nicosia, 1998, Am. J. Path. 153: 11-16). In one embodiment of the invention, the VEGF analog is a VEGF-C analog. VEGF-C analogs include "modified VEGF-C proteins", "VEGF-C analogs", "VEGF-C receptor antagonists", "VEGF-C chimeras", "VEGF-C fusion proteins" and "VEGF-C single chain molecules." A VEGF-C analog is a VEGF-C molecule containing at least one modified VEGF-C subunit.

VEGF-D is also produced as a propeptide (SEQ ID NO.: 52) that is proteolytically cleaved to form an active protein. VEGF-D is 48% identical to VEGF-C (Nicosia, supra). In one embodiment of the invention, the VEGF analog is a VEGF-D analog. VEGF-D analogs include "modified VEGF-D proteins", "VEGF-D analogs", "VEGF-D receptor antagonists", "VEGF-D chimeras", "VEGF-D fusion proteins" and "VEGF-D single chain molecules." A VEGF-D analog is a VEGF-D molecule containing at least one modified VEGF-D subunit.

Placenta growth factor (PIGF) exists in three isoforms, PIGF-1 (SEQ ID NO.: 54), PIGF-2 (SEQ ID NO.: 56) and PIGF-3 (SEQ ID NO.: 58). PIGF-2 contains an exon 6 encoded peptide which bestows heparin and neuropilin-1 binding properties absent in the other two isoforms. Both

PIGF-1 and PIGF-2 have been reported as being capable of inducing endothelial cell migration (Migdal et al., 1998, J. Biol. Chem. 273: 22272-22278). In one embodiment of the invention, the VEGF analog is a PIGF analog. In another embodiment, the VEGF analog is PIGF-1 or PIGF-2. PIGF analogs include "modified PIGF proteins", "PIGF analogs", "PIGF receptor antagonists", "PIGF chimeras", "PIGF fusion proteins" and "PIGF single chain molecules." PIGF analogs are PIGF molecules with at least one modified PIGF subunit.

The VEGF analogs of the invention are modified animal or human VEGF molecules. In one embodiment of the invention, the VEGF analogs are mammalian VEGF molecules. In another embodiment of the invention, the VEGF analogs are avian VEGF molecules. The VEGF analogs of the present invention include, but are not limited to, modified primate, canine, feline, bovine, equine, porcine, ovine, murine, rat and rabbit VEGF molecules. In one embodiment, the animal VEGF analog is a VEGF-A analog. For instance, the animal VEGF-A analog of the invention can be an animal VEGF₁₆₅ or VEGF_{165b} analog.

The modified VEGF molecules of species other than human have substitutions at positions corresponding to those in the modified human VEGF molecules disclosed herein and may be identified using any alignment program, including but not limited to DNASIS, ALIONment, SIM and GCG programs such as Gap, BestFit, FrameAlign, and Compare. As can be appreciated by one of skill in the art, the corresponding amino acid to be replaced with a basic amino acid may not be identical to the one in human VEGF-A. For instance, a skilled artisan would appreciate that a glutamate (E) may correspond to a different acidic amino acid in an animal such as aspartate (D).

In another embodiment, the corresponding amino acid is identified as being located in the same general position within a defined structure, for instance, on an outer loop structure. The structure of a protein can be predicted using software based on the amino acids of the protein. Accordingly, one of skill in the art can use software that predicts protein folding and loop structure to identify the corresponding position in a related protein.

Design of VEGF Receptor Antagonists

The VEGF receptor antagonists encompassed by the present invention may be designed by comparing the amino acid sequences of the VEGF of interest to that of other species to identify basic residues in the proteins of VEGF of other species. For instance, a VEGF-A molecule of instance can be designed by comparing a human VEGF-A to that of another species. Such methods are disclosed in U.S. Pat. No. 6,361,992, which is herein incorporated by reference in its entirety. Consideration may also be given to the relative biological activity of VEGF from various species as to which species to choose for comparison and amino acid substitution. Further homology modeling based on the structure of related glycoproteins is useful to identify surface-exposed amino acid residues. Homology modeling can be performed by methods generally known in the art, including, but not limited to, the use of protein modeling computer software.

The present invention also provides a modified VEGF protein, wherein the modified VEGF comprises an amino acid(s) substituted at a position(s) corresponding to the same amino acid position in a VEGF protein from another species having an increased binding affinity and/or decreased bioactivity over the wild-type VEGF. For example, snake venom VEGF-F binds to KDR with high affinity and strongly stimulates proliferation of vascular endothelial cells in vitro. One can compare human VEGF-A to snake venom VEGF, design human VEGF-A proteins with amino acid substitutions at one

or more positions where the snake venom and human sequences differ, construct human VEGF-A proteins with the selected changes, and administer the modified human VEGF-A to humans. Although snake venom VEGF-F demonstrates an increase in KDR binding affinity and bioactivity, i.e., binding affinity and bioactivity are correlated, compared to human VEGF, one of skill in the art would understand that amino acid substitutions could be empirically tested to identify amino acid substitutions which increase receptor binding affinity but decrease or have no effect on bioactivity. An amino acid substitution which increases receptor binding affinity and/or decreases or has no effect on bioactivity may then be combined with one or more other amino acid substitutions known to increase receptor binding affinity and/or decrease bioactivity.

In another embodiment of the invention, the modified VEGF molecule can contain one or more amino acids substituted at a position(s) corresponding to the same amino acid position in a VEGF homolog that naturally exists in arthropods. In arthropods, a single growth factor performs the tasks performed by PDGF and VEGF in higher organisms. One of skill in the art would understand that amino acid substitutions could be empirically tested to identify amino acid substitutions which increase receptor binding affinity but decrease or have no effect on bioactivity, or, alternatively, have little effect on receptor binding affinity but decrease bioactivity.

Further, the present invention provides a modified VEGF, wherein the modified VEGF comprises a basic amino acid(s) substituted at a position(s) corresponding to the same amino acid in a different VEGF or VEGF isoform or closely related glycoprotein such as proteins in the PDGF family from the same species or different species. For example, VEGF₁₆₅ can be compared to PDGF from the same species and amino acid substitutions made to the VEGF protein based on any sequence divergence. A skilled artisan can compare two or more sequences of VEGF proteins or VEGF-related proteins using methods known in the art such as the use of alignment software, including but not limited to, DNASIS, ALIONment, SIM and GCG programs such as Gap, BestFit, FrameAlign, and Compare.

In another aspect of the invention, the amino acid substitutions described herein can be incorporated into closely related proteins such as VEGF-E (SEQ ID NO.: 60), VEGF-F (SEQ ID NO.: 62) and PDGF (SEQ ID NO.: 63 and SEQ ID NO.: 64). For instance, one or more basic amino acid substitutions selected from the group consisting of E67, E72, E73, I83 and Q87 can be compared to a PDGF isoform from the same species and amino acid substitutions made to the PDGF isoform.

The VEGF analogs of the invention may be designed to display a decreased receptor binding affinity to Flt-1 receptors compared to wild-type VEGF-A. Although these analogs display a decreased receptor binding affinity to Flt-1, they may have an increased or comparable receptor binding affinity to KDR compared to wild-type VEGF-A.

The VEGF analogs of the invention may be designed to display a decreased receptor binding affinity to co-receptors, including, but not limited to, neuropilin-1 or neuropilin-2 compared to that of wild-type VEGF. Analogs with decreased receptor binding affinity to neuropilin-1 or neuropilin-2 may have increased or similar receptor binding affinity to KDR, Flt-1 or VEGFR3 compared to that of wild-type VEGF. For instance, VEGF-A analogs can be designed which exhibit decreased receptor binding affinity to neuropilin-1 and increased receptor binding affinity to KDR and/or Flt-1. In one embodiment of the invention, the VEGF-A displaying decreased receptor binding affinity to neuropilin-1 is an ana-

log designed in the VEGF_{165b} splice variant. In another embodiment, VEGF-B₁₆₇ and PlGF-2 analogs can be designed which exhibit decreased receptor binding affinity to neuropilin-1 and increased binding affinity to Flt-1.

In one embodiment of the invention, VEGF analogs are designed to exhibit decreased receptor binding affinity to neuropilin-1 or neuropilin-2 compared to wild-type VEGF by disrupting the VEGF neuropilin binding site. This can be accomplished by reducing the number of cysteine amino acid residues in the neuropilin-1 receptor binding domain. For instance, VEGF₁₆₅ analogs can be designed to disrupt the neuropilin 1 binding site in VEGF₁₆₅ by substituting the cysteine residues at positions 146 and/or 160 of SEQ ID NO.: 4 with amino acids such as serine which cause a disruption of the disulfide bridge. The substitution of cysteine residues at positions 146 and 160 of SEQ ID NO.: 4 disrupts neuropilin-1 binding but does not disrupt heparin binding. Mutations at positions 146 and/or 160 can be coupled with one or more mutations to increase, maintain or restore receptor binding affinity to KDR, Flt-1 and/or VEGFR3 as described herein.

Similarly, the present invention includes VEGF analogs which exhibit decreased receptor binding affinity to neuropilin-2 compared to wild-type VEGF. For instance, the invention includes VEGF-C and VEGF-D analogs which exhibit reduced binding affinity to neuropilin-2 but increased or similar binding affinity to KDR and/or VEGFR3 compared to wild-type VEGF-C or VEGF-D, respectively.

The invention also includes VEGF analogs which exhibit enhanced stability and resistance to proteases. In one embodiment, amino acids substitutions at positions A111 and A148 of SEQ ID NO.: 4 are incorporated in a VEGF-A analog to improve resistance to proteases. The invention also includes VEGF-C and VEGF-D analogs which contain mutations preventing the cleavage of the VEGF-C propeptide or VEGF-D propeptide, respectively. For instance, the present invention includes VEGF-C and VEGF-D analogs that contain one or more mutations which induce resistance to serine protease plasmin and/or other members of the plasminogen family.

In another embodiment of the invention, VEGF analogs which exhibit increased receptor binding affinity to one or more VEGF receptors, preferably KDR, can be created in a naturally occurring VEGF molecule which exhibits antagonistic properties. For instance, VEGF_{165b}, an isoform isolated from kidney tissue, can be modified to incorporate the amino acid substitutions associated with an increase in receptor binding ability and decrease in bioactivity of the protein. Similarly, a skilled artisan could incorporate the amino acid substitutions of the present invention in synthetic or new isoforms of VEGF which contain the properties of VEGF_{165b}. In particular, the mutations of the invention can be used with other VEGF proteins which contain the amino acids SLTRKD (SEQ ID NO.: 70), i.e., the amino acids coded for by what has been termed exon 9, in addition to or in place of the amino acids coded for by exon 8 (CDKPRR; SEQ ID NO.: 71).

Amino Acid Substitutions

The VEGF analogs of the present invention contain one or more basic amino acid substitutions which confer enhanced receptor binding affinity and decreased bioactivity. In one embodiment of the invention, the VEGF analogs are VEGF receptor antagonists, including but not limited to VEGF-A antagonists.

A modified VEGF molecule of the invention may have a basic amino acid substitution in one or more subunits, i.e., monomers, of VEGF. Basic amino acids comprise the amino acids lysine (K), arginine (R) and histidine (H), and any other basic amino acids which may be a modification of any of these three amino acids, synthetic basic amino acids not nor-

13

mally found in nature, or any other amino acids which are positively charged at a neutral pH. Preferred amino acids, among others, are selected from the group consisting of lysine and arginine.

In one embodiment, a modified VEGF molecule of the invention comprises at least one modified subunit, wherein the modified subunit comprises a basic amino acid substitution at position I83 of wild-type human VEGF₁₆₅ (SEQ ID NO.: 4), VEGF₁₂₁ (SEQ ID NO.: 6), VEGF₁₄₅ (SEQ ID NO.: 8), VEGF₁₄₈ (SEQ ID NO.: 10), VEGF_{165b} (SEQ ID NO.: 13), VEGF₁₈₃ (SEQ ID NO.: 15), VEGF₁₈₉ (SEQ ID NO.: 17) or VEGF₂₀₆ (SEQ ID NO.: 19). For instance, the invention includes an I83K amino acid substitution in SEQ ID NOs.: 4, 6, 8, 10, 13, 15, 17 or 19 corresponding to the amino acid sequences of VEGF-A isoforms.

The invention also includes a basic amino acid substitution in the position corresponding to position 83 in other VEGF molecules, i.e., VEGF-B, VEGF-C, VEGF-D and PlGF, such as position I83 of VEGF-B₁₆₇ (SEQ ID NO.: 48) or VEGF-B₁₈₆ (SEQ ID NO.: 50) and position 191 of PlGF-1 (SEQ ID NO.: 54), PlGF-2 (SEQ ID NO.: 56) or PlGF-3 (SEQ ID NO.: 58).

The invention includes modified VEGF molecules in animals other than humans, wherein the VEGF molecule contains, in one or more subunits, a basic amino acid substitution in the position corresponding to position 83 in human VEGF-A. In one embodiment, the modified animal VEGF is a modified VEGF-A molecule. For instance, the present invention includes a basic amino acid substitution at position I83 in primate (SEQ ID NO.: 22), position I82 in bovine (SEQ ID NO.: 25), position I82 in canine (SEQ ID NO.: 28), position I83 in chicken (SEQ ID NO.: 31), position I82 in equine (SEQ ID NO.: I82), position I82 in murine (SEQ ID NO.: 37), position I82 in porcine (SEQ ID NO.: 40), position I82 of rat (SEQ ID NO.: 43) and position I82 in ovine (SEQ ID NO.: 46).

The invention also envisions a modified VEGF-related protein, including, but not limited to VEGF-E, VEGF-F and PDGF, containing an amino acid substitution corresponding to position I83 of SEQ ID NO.: 4. For instance, VEGF-F (SEQ ID NO.: 62) can be modified to include an I83 amino acid substitution.

The modified VEGF molecule of the invention can contain basic amino acid substitutions which further increase the binding affinity or decrease bioactivity of VEGF compared to wild-type VEGF such as wild-type VEGF-A. VEGF molecules with basic amino acid substitutions at one or more of positions 44, 67, 72, 73 and/or 87 of VEGF₁₆₅ (SEQ ID NO.: 4), VEGF₁₂₁ (SEQ ID NO.: 6), VEGF₁₄₅ (SEQ ID NO.: 8), VEGF₁₄₈ (SEQ ID NO.: 10), VEGF_{165b} (SEQ ID NO.: 13), VEGF₁₈₃ (SEQ ID NO.: 15), VEGF₁₈₉ (SEQ ID NO.: 17) and VEGF₂₀₆ (SEQ ID NO.: 19) can increase binding affinity for KDR compared to wild-type VEGF. For instance, the invention includes the basic amino acid modifications E44R, E44K, E72R, E72K, E73R, E73K, Q87R, Q87K and E67K.

In one embodiment of the invention, basic amino substitutions corresponding to positions 44, 67, 72, 73 and/or 87 of SEQ ID NO.: 4 are coupled with the basic amino acid substitution corresponding to position 83 of SEQ ID NO.: 4 to produce a VEGF receptor antagonists. For instance, the modified amino acids of the present invention include basic amino acid substitutions at positions 72+73+83, 44+83, 72+83, 73+83, 44+72+83, 44+73+83, 44+72+73+83, 44+83+87, 83+87, 67+72+73+83, 44+67+83, 67+72+83, 67+73+83, 44+67+72+83, 44+67+73+83, 44+67+72+73+83, 44+67+83+87 and 67+83+87.

14

In another embodiment of the invention, the analog is a VEGF_{165b} molecule containing one or more basic amino acids at positions E44, E67, E72, E73 and Q87 and optionally a basic amino acid substitution at position I83. When the VEGF-A isoform is VEGF_{165b}, it is possible to generate a VEGF analog of the invention with increased binding affinity and decreased bioactivity compared to wild-type VEGF-A, including VEGF₁₆₅, by incorporating a single amino acid modification that would otherwise only result in an increase in receptor binding affinity in other VEGF₁₆₅.

As can be appreciated by a skilled artisan, the invention includes VEGF proteins and VEGF-related proteins other than VEGF-A that contain basic amino acid modifications corresponding to those of positions E44, E67, E72, E73 and/or Q87 of VEGF-A (SEQ ID NO.: 4). For instance, the invention includes a modified VEGF-B analog (SEQ ID NOs.: 48 and 50) containing one or more basic amino acid substitutions at positions A44, E67, G72, Q73 and S87 and a modified VEGF-F analog (SEQ ID NO.: 62) containing one or more basic amino acid substitutions at positions E44, E67, E72, E73 and Q87.

A modified animal, i.e., non-human, VEGF-A molecule of the invention can likewise contain additional amino acid modifications to increase binding affinity or decrease bioactivity of the modified animal VEGF molecule compared to wild-type animal VEGF. The invention includes the use of these modifications in conjunction with an amino acid substitution that corresponds to I83 of SEQ ID NO.: 4 as described above. For instance, the present invention includes one or more basic amino acid substitutions selected from the group of positions E44, E67, E72, E73, I83 and I87 of primate (long-tailed macaque) VEGF-A (SEQ ID NO.: 22); one or more basic amino acid substitutions selected from the group of positions E43, E66, E71, E72, I82 and Q86 of bovine VEGF-A (SEQ ID NO.: 25); one or more basic amino acid substitutions selected from the group of positions E43, E66, E71, E72, I82 and Q86 of canine VEGF-A (SEQ ID NO.: 28); one or more basic amino acid substitutions selected from the group of positions E44, E67, D72, V73, I83 and Q87 of avian (chicken) VEGF-A (SEQ ID NO.: 31); one or more basic amino acid substitutions selected from the group of positions E43, E66, A71, E72, I82 and Q86 of equine VEGF-A (SEQ ID NO.: 34); one or more basic amino acid substitutions selected from the group of positions E43, E66, S71, E72, I82 and Q86 of murine VEGF-A (SEQ ID NO.: 37); one or more basic amino acid substitutions selected from the group of positions E43, E66, E71, E72, I82 and Q86 of porcine VEGF-A (SEQ ID NO.: 40); one or more basic amino acid substitutions selected from the group of positions E43, E66, S71, E72, I82 and Q86 of rat VEGF-A (SEQ ID NO.: 43); and one or more basic amino acid substitutions selected from the group of positions E43, E66, E71, E72, I82 and Q86 of ovine VEGF-A (SEQ ID NO.: 46).

VEGF analogs containing one or more basic amino acid substitutions can also be combined with amino acid substitutions designed to disrupt a co-receptor binding site. In one embodiment, the VEGF analogs of the invention contain a disrupted neuropilin-1 binding site. The neuropilin-1 binding site comprises amino acids 111 to 165 of VEGF₁₆₅ (SEQ ID NO.: 04). This domain overlaps the heparin binding domain encoded by exons 6 and 7. The invention includes any amino acid modifications in or near (i.e., within about 5 amino acids) that disrupt the neuropilin-1 binding site domain but which do not disrupt the ability of the heparin binding domain to bind heparin sulfate. Such amino acid modifications can be determined empirically by a skilled artisan.

In another embodiment of the invention, the basic amino acid substitutions of the invention are coupled with one or more amino acid substitutions that enhance stability and increase serum half-life by eliminating one or more proteolytic cleavage sites. In one embodiment, the additional amino acid substitutions reduce proteolytic cleavage. In another embodiment, the additional amino acid substitutions prevent proteolytic cleavage. The invention includes VEGF analogs that contain one or more mutations which induce resistance to plasmin and other members of the plasminogen family. In one embodiment of the invention, at least one subunit of a VEGF molecule contains an amino acid substitution corresponding to amino acid positions A111 and/or A148 such as A111P and/or A148P of VEGF₁₆₅ (SEQ ID NO.: 4) or VEGF_{165b} (SEQ ID NO.: 13). For instance, the invention includes VEGF₁₂₁, VEGF₁₄₅, VEGF₁₄₈, VEGF₁₈₃, VEGF₁₈₉ and VEGF₂₀₆ containing an amino acid substitution at position A111. The invention includes one or more mutations in VEGF-B, VEGF-C, VEGF-D and PlGF which inhibit

or reduce protease cleavage. For instance, the invention includes amino acid substitutions which prevent the cleavage of VEGF-C and VEGF-D necessary for bioactivity.

In another embodiment, half-life can be increased by linking VEGF monomers and by constructing fusion proteins. Increasing the size of a VEGF analog without interfering with binding sites can increase the half-life of the molecule.

Increased half-life may be provided by crosslinking, including but not limited to pegylation or conjugation of other appropriate chemical groups. Such methods are known in the art, for instance as described in U.S. Pat. No. 5,612,034, U.S. Pat. No. 6,225,449, and U.S. Pat. No. 6,555,660, each of which is incorporated by reference in its entirety. Half-life may also be increased by increasing the number of negatively charged residues within the molecule, for instance, the number of glutamate and/or aspartate residues. Such alteration may be accomplished by site directed mutagenesis or by an insertion of an amino acid sequence containing one or more negatively charged residues into said modified VEGF, including insertions selected from the group consisting of GEFT and GEFTT, among others.

The half-life of a protein is a measurement of protein stability and indicates the time necessary for a one-half reduction in the concentration of the protein. The serum half-life of the modified VEGF molecules described herein may be determined by any method suitable for measuring VEGF levels in samples from a subject over time, for example, but not limited to, immunoassays using anti-VEGF antibodies to measure VEGF levels in serum samples taken over a period of time after administration of the modified VEGF, or by detection of labeled VEGF molecules, i.e., radiolabeled molecules, in samples taken from a subject after administration of the labeled VEGF.

The rate of absorption of a VEGF analog of the present invention may result in increased or decreased duration of action. A VEGF analog with an increased rate of absorption and decreased duration of action may be beneficial for patients receiving a VEGF analog pharmaceutical composition by way of subcutaneous administration or other route of administration generally associated with a slow rate of absorption and/or increased duration of action by counteracting the absorption qualities associated with the route of administration.

Linker

The VEGF analog of the invention can contain two or more monomers separated by a linker peptide. A linker peptide can be used to form a VEGF analog in a single chain conformation. A skilled artisan can appreciate that various types of linkers can be used in the present invention to form a VEGF single chain molecule that is capable of binding a VEGF receptor and which acts as a VEGF receptor antagonist. A linker peptide should not hinder the ability of the single chain molecule to bind a VEGF receptor.

The linker peptide can range from about 2 to about 50 or more amino acids in length. For instance, the linker can consist of about 2 amino acids, about 3 amino acids, about 4 amino acids, about 5 amino acids, about 6 amino acids, about 7 amino acids, about 8 amino acids, about 9 amino acids, about 10 amino acids, about 10-15 amino acids, or about 15-20 amino acids. In one embodiment of the invention, the linker is Gly-Ser or contains Gly-Ser. In another embodiment, the linker is a glycine-rich polypeptide chain.

VEGF molecules containing a linker can be constructed using the methods described herein. A skilled artisan would be able to appreciate that VEGF analog molecules of the invention containing linker peptides can include any of the mutations described herein, in one or more monomers. Fur-

ther, a VEGF analog containing one or more linker peptides can link more than one type of VEGF protein or isoform. For instance, the present invention includes, but is not limited to, a modified VEGF single chain molecule with a wild-type VEGF₁₆₅ monomer linked to a modified VEGF₁₆₅ monomer containing an I83B substitution; a wild-type VEGF₁₆₅ monomer linked to a modified VEGF_{165b} containing an I83B substitution; and a modified VEGF₁₆₅ monomer fused to a modified VEGF-F monomer.

VEGF Fusion Proteins

The present invention also includes fusion proteins, i.e., chimeras, containing one or more modified VEGF proteins or fragments. "Fusion protein" and "chimera" are used interchangeably herein. As used herein, a VEGF moiety is a VEGF protein or protein fragment containing one or more of the basic amino acid substitutions of the invention. A VEGF fusion protein can have one or more VEGF moieties.

Such a fusion protein may be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the fusion protein by any of the means described herein. Alternatively, such a fusion protein may be made by protein synthesis techniques, for example, using a peptide synthesizer.

The fusion protein of the invention contains at least one VEGF protein or protein fragment containing one or more basic amino acid substitutions described herein. In one embodiment the fusion protein contains a basic amino acid substitution at position I83 of VEGF₁₆₅ (SEQ ID NO. 4), VEGF_{165b} (SEQ ID NO. 13), VEGF₁₂₁ (SEQ ID NO.: 6), VEGF₁₄₅ (SEQ ID NO.: 8), VEGF₁₄₈ (SEQ ID NO.: 10), VEGF₁₈₃ (SEQ ID NO.: 15), VEGF₁₈₉ (SEQ ID NO.: 17) or VEGF₂₀₆ (SEQ ID NO.: 19). In another embodiment, the fusion protein contains at least one basic amino acid substitution at a position corresponding to I83K of SEQ ID NO.: 4 in another VEGF protein, for instance, an isoform of VEGF-B, VEGF-C, VEGF-D or PlGF. As can be appreciated by a skilled artisan, human or animal VEGF proteins or fragments thereof may be used for the fusion proteins of the invention.

In one embodiment of the invention, two different VEGF protein subunits or fragments thereof are fused. For instance, the invention includes a VEGF-A subunit or fragment thereof fused to a VEGF-B subunit or fragment thereof, a VEGF-C subunit or fragment thereof, a VEGF-D subunit or fragment thereof, or a PlGF subunit or fragment thereof; a VEGF-B subunit or fragment thereof fused to a VEGF-A subunit or fragment thereof, a VEGF-C subunit or fragment thereof, a VEGF-D subunit or fragment thereof, or a PlGF subunit or fragment thereof; a VEGF-C subunit or fragment thereof fused to a VEGF-A subunit or fragment thereof, a VEGF-B subunit or fragment thereof, a VEGF-D subunit or fragment thereof, or a PlGF subunit or fragment thereof; a VEGF-D subunit or fragment thereof fused to a VEGF-A subunit or fragment thereof, a VEGF-B subunit or fragment thereof, a VEGF-C subunit or fragment thereof, or a PlGF subunit or fragment thereof; and a PlGF subunit or fragment thereof fused to a VEGF-A subunit or fragment thereof, a VEGF-B subunit or fragment thereof, a VEGF-C subunit or fragment thereof, or a VEGF-D subunit or fragment thereof.

The invention includes fusion proteins comprised of two or more different isoforms of the same VEGF protein or fragments thereof. For instance, the invention includes a fusion protein comprised of a VEGF₁₆₅ subunit or fragment thereof fused to a VEGF₁₂₁ subunit or fragment thereof, a VEGF₁₄₅ subunit or fragment thereof, a VEGF₁₄₈ subunit or fragment thereof, a VEGF_{165b} subunit or fragment thereof, a VEGF₁₈₃ subunit or fragment thereof, a VEGF₁₈₉ subunit or fragment thereof, or a VEGF₂₀₆ subunit or fragment thereof.

The invention also includes a VEGF_{165b} subunit or fragment thereof fused to a VEGF₁₂₁ subunit or fragment thereof, a VEGF₁₄₅ subunit or fragment thereof, a VEGF₁₄₈ subunit or fragment thereof, a VEGF₁₆₅ subunit or fragment thereof, a VEGF₁₈₃ subunit or fragment thereof, a VEGF₁₈₉ subunit or fragment thereof, or a VEGF₂₀₆ subunit or fragment thereof.

The basic amino acid substitutions of the invention may be present in one or more subunits of the protein. For example, a fusion protein containing a VEGF₁₆₅ subunit and VEGF_{165b} subunit may only contain an amino acid substitution in the VEGF₁₆₅ subunit. The invention includes a wild-type VEGF₁₆₅ subunit fused by way of a GS linker to a VEGF₁₆₅ containing an I83K amino acid substitution. As can be appreciated by one of skill in the art, the fusion proteins of the present invention containing one mutated subunit can be created in both orientations, i.e., the subunit containing the mutation can be at either the N- or C-terminus of the fusion protein.

In another embodiment of the invention, a VEGF subunit or fragment thereof is fused to a related protein subunit or fragment thereof. For instance, a VEGF subunit or fragment thereof can be fused to a PDGF subunit or other glycoprotein subunit or fragment thereof.

As can be appreciated by one of ordinary skill in the art, the fusion proteins described herein can be constructed using human or animal VEGF sequences. Further, a fusion protein can be constructed using a human VEGF subunit fused to an animal VEGF subunit.

A VEGF fusion protein should be understood to be a VEGF analog. All modifications disclosed herein, for instance, modifications to further increase receptor binding affinity, modifications to increase half-life and stability, modifications to reduce or inhibit protease cleavage, and modifications to disrupt a co-receptor binding site such as a neuropilin-1 binding site can be incorporated in one or more subunits of the VEGF fusion protein.

The fusion proteins of the invention can also contain a linker separating the two or more VEGF subunits or VEGF-related protein subunits. The linker can be covalently linked to and between the peptides of the fusion protein.

VEGF and Toxin Fusion Proteins

The present invention provides fusion proteins comprising a toxin and one or more modified VEGF subunits, i.e., monomers, containing one or more of the basic amino acid substitutions described herein. For instance, the VEGF monomer, i.e., subunit, of a VEGF-toxin fusion protein can contain a basic amino acid at one or more amino acid positions corresponding to the amino acid positions from the group consisting of 44, 67, 72, 73, 83 and 87 (SEQ ID NO.: 4 or SEQ ID NO.: 13). The VEGF and toxin fusion proteins of the invention may optionally contain a linker sequence separating the toxin and one or more VEGF subunits.

As used herein, the term "toxin" refers to a poisonous substance of biological origin. The toxin of the invention may be a soluble toxin as known in the art. The fusion proteins comprising a soluble toxin may be used to target tumors. Such fusion proteins may also be used for diagnostic purposes.

Examples of toxins include, but are not limited to, *Pseudomonas* exotoxins (PE), Diphtheria toxins (DT), ricin toxin, abrin toxin, anthrax toxins, shiga toxin, botulism toxin, tetanus toxin, cholera toxin, maitotoxin, palytoxin, ciguatera toxin, textilotoxin, batrachotoxin, alpha conotoxin, taipoxin, tetrodotoxin, alpha tityustoxin, saxitoxin, anatoxin, microcystin, aconitine, exfoliatin toxins A and B, enterotoxins, toxic shock syndrome toxin (TSST-1), *Y. pestis* toxin, gas gangrene toxin, and others.

In one embodiment, the present invention provides a pharmaceutical composition comprising a soluble toxin fused to a modified VEGF and a pharmaceutically acceptable carrier. In another embodiment, the present invention provides the use of a modified VEGF fusion protein comprising a soluble toxin for the manufacture of a medicament for the treatment or prevention of diseases or conditions associated with angiogenesis.

Without wishing to be bound by a theory, it is believed that the VEGF-toxin fusion protein of the invention prevents or reduces angiogenesis, the growth of tumors and/or the spread of cancer by targeting and killing the VEGF receptor and surrounding endothelial and tumor cells.

Expression and/or Synthesis of VEGF Receptor Antagonists

The present invention includes nucleic acids encoding the modified VEGF proteins of the invention, as well as vectors and host cells for expressing the nucleic acids.

As used herein, the terms "nucleic acid" or "polynucleotide" refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single or double stranded form. The invention includes a nucleic acid molecule which codes for a modified VEGF molecule of the invention. For instance, the invention includes a nucleic acid molecule that codes for a modified VEGF₁₆₅ molecule. The nucleic acid molecule of SEQ ID NO.: 1 which codes for wild-type VEGF₁₆₅ can be mutated by methods known in the art such that the mutated VEGF₁₆₅ nucleic acid molecule codes for the modified protein. Similarly, the nucleic acid molecule of SEQ ID NO.: 11 which codes for wild-type VEGF_{165b} can be mutated by methods known in the art such that it codes for a VEGF_{165b} molecule of the invention.

Once a nucleic acid encoding a particular modified VEGF of interest, or a region of that nucleic acid encoding a portion of the protein containing a basic amino acid substitution, is constructed, modified, or isolated, that nucleic acid can then be cloned into an appropriate vector, which can direct the in vivo or in vitro synthesis of the modified VEGF protein. Alternatively, the nucleic acid encoding a VEGF analog of the invention may be cloned or modified directly in the expression vector of interest. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted gene, or hybrid gene. These functional elements include, but are not limited to, a promoter, regions upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, an origin of replication, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the vector or the vector containing the insert, RNA splice junctions, a transcription termination region, or any other region which may serve to facilitate the expression of the inserted gene or hybrid gene. (See generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed. (1989)). Appropriate promoters for the expression of nucleic acids in different host cells are well known in the art, and are readily interchanged depending on the vector-host system used for expression. Exemplary vectors and host cells are described in U.S. Pat. No. 6,361,992, which is herein incorporated by reference in its entirety.

There are numerous *E. coli* (*Escherichia coli*) expression vectors known to one of ordinary skill in the art which are useful for the expression of the nucleic acid insert. Other vectors suitable for use include expression vectors from bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. These expression vectors will typically contain

expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences for example, for initiating and completing transcription and translation. If necessary, an amino terminal methionine can be provided by insertion of a Met codon 5' and in-frame with the downstream nucleic acid insert. Also, the carboxy-terminal extension of the nucleic acid insert can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression systems can be used. There are several advantages to yeast expression systems. First, evidence exists that proteins produced in a yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the MF¹-1 gene) is routinely used to direct protein secretion from yeast. (Brake, et al., "varies-Factor-Directed Synthesis and Secretion of Mature Foreign Proteins in *Saccharomyces cerevisiae*." Proc. Nat. Acad. Sci., 81:4642-4646 (1984)). The leader region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the KEX2 gene. This enzyme cleaves the precursor protein on the carboxyl side of a Lys-Arg dipeptide cleavage signal sequence. The VEGF coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The nucleic acid coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the nucleic acid coding sequences can be fused to a second protein coding sequence, such as Sj26 or beta-galactosidase, which may be used to facilitate purification of the fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast. Efficient post-translational glycosylation and expression of recombinant proteins can also be achieved in Baculovirus systems.

Mammalian cells permit the expression of proteins in an environment that favors important post-translational modifications such as folding and cysteine pairing, addition of complex carbohydrate structures, and secretion of active protein. Vectors useful for the expression of active proteins in mammalian cells are characterized by insertion of the protein coding sequence between a strong viral or other promoter and a polyadenylation signal. The vectors can contain genes conferring hygromycin resistance, gentamicin resistance, or other genes or phenotypes suitable for use as selectable markers, or methotrexate resistance for gene amplification. The chimeric protein coding sequence can be introduced into a Chinese hamster ovary (CHO) cell line using a methotrexate resistance-encoding vector, or other cell lines using suitable selection markers. Presence of the vector DNA in transformed cells can be confirmed by Southern blot analysis. Production of RNA corresponding to the insert coding sequence can be confirmed by Northern blot analysis. A number of other suitable host cell lines capable of secreting intact human proteins have been developed in the art, and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, etc. Expression vectors for these cells can include

expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Exemplary expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, etc. The vectors containing the nucleic acid segments of interest can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transformation is commonly utilized for prokaryotic cells, whereas calcium phosphate, DEAE dextran, or lipofectin mediated transfection or electroporation may be used for other cellular hosts.

Expression of the gene or hybrid gene can be either in vivo or in vitro. In vivo synthesis comprises transforming prokaryotic or eukaryotic cells that can serve as host cells for the vector. For instance, techniques for transforming fungi are well known in the literature, and have been described, for instance, by Beggs (ibid.), Hinnen et al. (Proc. Natl. Acad. Sci. USA 75: 1929-1933, 1978), Yelton et al., (Proc. Natl. Acad. Sci. USA 81: 1740-1747, 1984), and Russell (Nature 301: 167-169, 1983). Other techniques for introducing cloned DNA sequences into fungal cells, such as electroporation (Becker and Guarente, Methods in Enzymol. 194: 182-187, 1991) may be used. The genotype of the host cell will generally contain a genetic defect that is complemented by the selectable marker present on the expression vector. Choice of a particular host and selectable marker is well within the level of ordinary skill in the art.

Cloned DNA sequences comprising modified VEGF and VEGF fusion proteins of the invention may be introduced into cultured mammalian cells by, for example, calcium phosphate-mediated transfection (Wigler et al., Cell 14: 725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7: 603, 1981; Graham and Van der Eb, Virology 52: 456, 1973.) Other techniques for introducing cloned DNA sequences into mammalian cells, such as electroporation (Neumann et al., EMBO J. 1: 841-845, 1982), or lipofection may also be used. In order to identify cells that have integrated the cloned DNA, a selectable marker is generally introduced into the cells along with the gene or cDNA of interest. Preferred selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. A preferred amplifiable selectable marker is the DHFR gene. A particularly preferred amplifiable marker is the DHFR^r (see U.S. Pat. No. 6,291,212) cDNA (Simonsen and Levinson, Proc. Natl. Acad. Sci. USA 80: 2495-2499, 1983). Selectable markers are reviewed by Thilly (Mammalian Cell Technology, Butterworth Publishers, Stoneham, Mass.) and the choice of selectable markers is well within the level of ordinary skill in the art.

Alternatively, expression of the gene can occur in an in vitro expression system. For example, in vitro transcription systems are commercially available which are routinely used to synthesize relatively large amounts of mRNA. In such in vitro transcription systems, the nucleic acid encoding the modified VEGF would be cloned into an expression vector adjacent to a transcription promoter. For example, the Bluescript II cloning and expression vectors contain multiple cloning sites which are flanked by strong prokaryotic transcription promoters. (Stratagene Cloning Systems, La Jolla, Calif.). Kits are available which contain all the necessary reagents for in vitro synthesis of an RNA from a DNA template such as the Bluescript vectors. (Stratagene Cloning Systems, La Jolla, Calif.). RNA produced in vitro by a system

such as this can then be translated in vitro to produce the desired VEGF analog (Stratagene Cloning Systems, La Jolla, Calif.).

Another method of producing a VEGF receptor antagonist is to link two peptides or polypeptides together by protein chemistry techniques. Peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert-butyloxycarbonyl) chemistry. (Applied Biosystems, Inc., Foster City, Calif.). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to a hybrid VEGF protein can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a hybrid peptide can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form a hybrid peptide. (Grant, G. A., "Synthetic Peptides: A User Guide," W. H. Freeman and Co., N.Y. (1992) and Bodansky, M. and Trost, B., Ed., "Principles of Peptide Synthesis," Springer-Verlag Inc., N.Y. (1993)). Alternatively, the peptide or polypeptide can be independently synthesized in vivo as described above. Once isolated, these independent peptides or polypeptides may be linked to form a VEGF via similar peptide condensation reactions. For example, enzymatic or chemical ligation of cloned or synthetic peptide segments can allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen, L., et al., *Biochemistry*, 30:4151 (1991); Dawson, et al., "Synthesis of Proteins by Native Chemical Ligation," *Science*, 266:776-779 (1994)).

The invention also provides fragments of modified VEGF which have antagonist activity. The polypeptide fragments of the present invention can be recombinant proteins obtained by cloning nucleic acids encoding the peptides in an expression system capable of producing the peptides. For example, amino or carboxy-terminal amino acids can be sequentially removed from either the native or the VEGF protein and the respective activity tested in one of many available assays described above. In another example, the modified proteins of the invention may have a portion of either amino terminal or carboxy terminal amino acids, or even an internal region of the protein, replaced with a polypeptide fragment or other moiety, such as biotin, which can facilitate in the purification of the modified VEGF. For example, a modified VEGF can be fused to a maltose binding protein, through either peptide chemistry of cloning the respective nucleic acids encoding the two polypeptide fragments into an expression vector such that the expression of the coding region results in a hybrid polypeptide. The hybrid polypeptide can be affinity purified by passing it over an amylose affinity column, and the modified VEGF can then be separated from the maltose binding region by cleaving the hybrid polypeptide with the specific protease factor Xa. (See, for example, New England Biolabs Product Catalog, 1996, pg. 164).

The VEGF analog of the invention can be a heterodimer or a homodimer. In one embodiment, the VEGF analog is a fusion protein containing one or more VEGF subunits. The VEGF fusion protein of the invention can be a single chain protein containing two or more VEGF subunits separated by linking peptides. In another embodiment, the VEGF analog of the invention is a fusion protein containing one or more VEGF

subunits fused to a toxin. The VEGF analog and VEGF analog fusion protein of the invention can be isolated and purified by means known in the art.

All of the VEGF analogs of the invention contain at least one basic amino acid substitution in at least one VEGF subunit. In one embodiment of the invention, the VEGF analogs of the invention contain at least two basic amino acid substitutions, at least 3 basic amino acid substitutions, at least 4 basic amino acid substitutions or at least 5 basic amino acid substitutions in at least one or at least two VEGF subunits.

The invention includes VEGF analogs containing VEGF active fragments, i.e., peptides that are not full length proteins. Active fragments of the modified VEGF of the invention can also be synthesized directly or obtained by chemical or mechanical disruption of larger modified VEGF protein. An active fragment is defined as an amino acid sequence of at least about 5 consecutive amino acids, at least 10 consecutive amino acids, at least 20 consecutive amino acids, at least 30 consecutive amino acids, at least 40 consecutive amino acids, at least 50 consecutive amino acids, at least 60 consecutive amino acids, at least 70 consecutive amino acids, at least 80 consecutive amino acids, at least 90 consecutive amino acids, at least 100 consecutive amino acids, at least 110 consecutive amino acids, at least 120 consecutive amino acids, at least 130 consecutive amino acids, at least 140 consecutive amino acids, at least 150 consecutive amino acids, or at least 160 consecutive amino acids derived from the natural amino acid sequence, which has the relevant activity, e.g., binding or regulatory activity. The fragments, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the peptide is not significantly altered or impaired compared to the modified VEGF. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its biol longevity and/or bioactivity, etcetera. In any case, the peptide must possess a bioactive property, such as binding activity, regulation of binding at the binding domain, etcetera. Functional or active regions of the VEGF may be identified by mutagenesis of a specific region of the hormone, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the receptor (Zoller, M. J. et al.).

Methods of Use

The invention encompasses methods for reducing VEGF-mediated angiogenesis, comprising contacting a cell expressing kinase domain receptor (KDR) with the VEGF analogs, including VEGF-A₁₆₅ and VEGF-A_{165b} analogs, described herein such that VEGF-mediated angiogenesis is reduced. KDR-expressing cells to be targeted by the methods of the invention can include either or both prokaryotic and eukaryotic cells. Such cells may be maintained in vitro, or they may be present in vivo, for instance in a patient or subject diagnosed with cancer or another angiogenesis-related disease.

The present invention includes methods of treating a patient diagnosed with an angiogenesis-related disease or condition with a therapeutically effective amount of any of the VEGF receptor antagonists described herein, comprising administering said VEGF analog or fusion protein to said patient such that said angiogenesis-related disease or condition is reduced or inhibited. In order to measure the reduction of angiogenesis, the patient's results may be compared to that of a patient administered a placebo. Exemplary angiogenesis-related diseases are described throughout this application, and include but are not limited to diseases selected from the

group consisting of tumors and neoplasias, hemangiomas, rheumatoid arthritis, osteoarthritis, septic arthritis, asthma, atherosclerosis, idiopathic pulmonary fibrosis, vascular restenosis, arteriovenous malformations, meningioma, neovascular glaucoma, psoriasis, Kaposi's Syndrome, angiofibroma, hemophilic joints, hypertrophic scars, Osler-Weber syndrome, pyogenic granuloma, retrolental fibroplasias, scleroderma, trachoma, von Hippel-Lindau disease, vascular adhesion pathologies, synovitis, dermatitis, endometriosis, pterygium, diabetic retinopathy, neovascularization associated with corneal injury or grafts, wounds, sores, and ulcers (skin, gastric and duodenal).

A patient suffering from a disease caused by or exacerbated by an increase in angiogenesis, a decrease in angiogenesis, or otherwise dysregulated angiogenesis can be treated with a VEGF analog alone or in combination with a known VEGF receptor antagonist, an anti-angiogenesis therapy, an anti-cancer therapy, or other therapy known to treat the disease or condition. As used herein, "therapy" includes but is not limited to a known drug. Known VEGF receptor antagonists or anti-angiogenesis therapies include but are not limited to agents that either interrupt VEGF/KDR interaction and/or block the KDR signal transduction pathway such as peptides that block binding of VEGF to KDR, antibodies to VEGF, antibodies to KDR, soluble receptors, tyrosine kinase inhibitors, anti-VEGF immunotoxins, ribozymes, antisense mediated VEGF suppression, and undersulfated, low molecular weight glycol-split heparin.

If a VEGF analog of the invention is used in combination with another therapy, the coupling of the therapies results in a synergistic effect. In addition, a VEGF analog of the present invention can be combined with a drug associated with an undesirable side effect. By coupling a VEGF analog with such a drug, the effective dosage of the drug with the side effect can be lowered to reduce the probability of the side effect from occurring.

The invention includes methods of treating a patient diagnosed with cancer with a therapeutically effective amount of any of the VEGF receptor antagonists described herein, comprising administering said antagonist to said patient such that the spread of said cancer is reduced or inhibited, i.e., metastasis is reduced or inhibited. The invention includes methods of treating a patient diagnosed with cancer with a therapeutically effective amount of any of the VEGF receptor antagonists described herein, comprising administering said antagonist to said patient such that the growth of a tumor is reduced or inhibited. In one embodiment, the VEGF analog functions by inhibiting angiogenesis by reducing or preventing VEGF-induced angiogenesis. In another embodiment, the VEGF analog is a VEGF-toxin fusion protein that prevents or reduces angiogenesis by targeting or killing tumor cells, vascular cells such as endothelial cells and/or VEGF receptors.

Cancers treatable by the methods of the present invention include all solid tumor and metastatic cancers, including but not limited to those selected from the group consisting of bladder, breast, liver, bone, kidney, colon, ovarian, prostate, pancreatic, lung, brain and skin cancers. The invention includes but is not limited to treatment of cancer with a VEGF analog of the present invention, alone, in combination with chemotherapy, or in combination with radiation therapy by methods known in the art (see U.S. Pat. No. 6,596,712). For instance, a VEGF analog may be used with cesium, iridium, iodine, or cobalt radiation.

The present invention includes methods of treating a patient diagnosed with infertility with a therapeutically effective amount of any of the VEGF receptor antagonists described herein, comprising administering said antagonist to

said patient such that infertility is deemed treated by one of skill in the art. Infertility can be measured by quantitative and qualitative parameters known in the art such as quantity of oocytes, fertilization rate, blastocyst formation rate, and embryo formation rate. Such infertility diseases include any disease associated with the expression of VEGF that compromises a patient's fertility including but not limited to unexplained female infertility, endometriosis, and unexplained male infertility. The invention includes but is not limited to treatment of infertility by administration of a VEGF analog alone or in combination with other anti-VEGF treatments, anti-angiogenesis treatments, and/or infertility treatments.

The present invention also includes methods of treating a patient diagnosed with an angiogenesis-associated eye disease with a therapeutically effective amount of any of the VEGF receptor antagonists described herein, comprising administering said antagonist to said patient such that said eye disease is reduced or inhibited. Such eye diseases include any eye disease associated with abnormal intraocular neovascularization, including but not limited to retinopathy of prematurity, diabetic retinopathy, retinal vein occlusion, and age-related macular degeneration. The invention includes but is not limited to treatment of angiogenesis-related eye diseases by administration of a VEGF analog alone or in combination with other anti-VEGF treatments, anti-angiogenesis treatments, and/or other eye disease treatments. For example, a VEGF analog of the present invention could be administered to a patient in conjunction with Pfizer's Macugen (pegaptanib) which is a pegylated anti-VEGF aptamer which acts by binding to and inhibiting the activity of VEGF for the treatment of diabetic macular edema, retinal vein occlusion, and age-related macular degeneration.

The present invention also includes methods of treating a patient diagnosed with an angiogenesis-associated inflammatory condition or autoimmune disease with a therapeutically effective amount of any of the VEGF receptor antagonists described herein, comprising administering said antagonist to said patient such that said inflammatory condition is reduced or inhibited. Such inflammatory conditions or diseases include any inflammatory disorder associated with expression of VEGF and activation of cells by VEGF, including but not limited to all types of arthritis and particularly rheumatoid arthritis and osteoarthritis, asthma, pulmonary fibrosis and dermatitis. The invention includes but is not limited to treatment of angiogenesis-related inflammatory conditions or autoimmune disease by administration of a VEGF analog alone or in combination with other anti-VEGF treatments, anti-angiogenesis treatments, inflammation therapeutics, and/or autoimmune disease therapeutics.

In another embodiment of the present invention, the modified VEGF protein of the invention is used as a diagnostic. The VEGF analogs of the invention or VEGF receptors can be displayed on a synthetic surface, such as in a protein or peptide array. Such an array is well known in the art and can be used to screen for VEGF analogs which bind to KDR and other receptors known to be involved in angiogenesis. The VEGF analogs disclosed herein can be used as positive controls to assess the ability of putative VEGF analogs to bind to KDR and other receptors known to be involved in angiogenesis. The invention also includes an array comprising the VEGF analogs of the present invention to screen for putative VEGF receptors which may be involved in angiogenesis.

Assays suitable for characterizing the analogs described herein are described in PCT/US/99/05908, which is herein incorporated by reference in its entirety. For instance, various immunoassays may be used including but not limited to competitive binding assays and non-competitive assay systems

using techniques such as radioimmunoassays, ELISA, sandwich immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, in situ immunoassays, western blots, precipitation reactions, agglutination assays, complement fixation assays, immunofluorescence assays, Protein A assays, and immunoelectrophoresis assays, etcetera.

Pharmaceutical Formulations

The invention provides methods of diagnosis and treatment by administration to a subject of an effective amount of a therapeutic of the invention. The subject may be an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, a non-human mammal is the subject.

The pharmaceutical compositions of the invention comprise an effective amount of one or more modified VEGF proteins of the present invention in combination with the pharmaceutically acceptable carrier. The compositions may further comprise other known drugs suitable for the treatment of the particular disease being targeted. An effective amount of the VEGF receptor antagonist of the present invention is that amount that blocks, inhibits or reduces VEGF stimulation of endothelial cells compared to that which would occur in the absence of the compound; in other words, an amount that decreases the angiogenic activity of the endothelium, compared to that which would occur in the absence of the compound. The effective amount (and the manner of administration) will be determined on an individual basis and will be based on the specific therapeutic VEGF receptor antagonist being used and a consideration of the subject (size, age, general health), the condition being treated (cancer, arthritis, eye disease, etc.), the severity of the symptoms to be treated, the result sought, the specific carrier or pharmaceutical formulation being used, the route of administration, and other factors as would be apparent to those skilled in the art. The effective amount can be determined by one of ordinary skill in the art using techniques as are known in the art. Therapeutically effective amounts of the compounds described herein can be determined using in vitro tests, animal models or other dose-response studies, as are known in the art. The VEGF proteins of the present invention can be used alone or in conjunction with other therapies. The therapeutically effective amount may be reduced when a VEGF analog is used in conjunction with another therapy.

The pharmaceutical compositions of the invention may be prepared, packaged, or sold in formulations suitable for intradermal, intravenous, subcutaneous, oral, rectal, vaginal, parenteral, intraperitoneal, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal, epidural or another route of administration. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. For example, the pharmaceutical compositions of the invention can be administered locally to a tumor via microinfusion. Further, administration may be by a single dose or a series of doses.

For pharmaceutical uses, the VEGF analogs of the present invention may be used in combination with a pharmaceutically acceptable carrier, and can optionally include a pharmaceutically acceptable diluent or excipient. Further, the VEGF analogs of the present invention may be used in combination

therapies, infertility therapies, autoimmune disease therapies, inflammation therapies, ocular disease therapies, and skin disease therapies.

The present invention thus also provides pharmaceutical compositions suitable for administration to a subject. The carrier can be a liquid, so that the composition is adapted for parenteral administration, or can be solid, i.e., a tablet or pill formulated for oral administration. Further, the carrier can be in the form of a nebulizable liquid or solid so that the composition is adapted for inhalation. When administered parenterally, the composition should be pyrogen free and in an acceptable parenteral carrier. Active compounds can alternatively be formulated or encapsulated in liposomes, using known methods. Other contemplated formulations include projected nanoparticles and immunologically based formulations.

Liposomes are completely closed lipid bilayer membranes which contain entrapped aqueous volume. Liposomes are vesicles which may be unilamellar (single membrane) or multilamellar (onion-like structures characterized by multiple membrane bilayers, each separated from the next by an aqueous layer). The bilayer is composed of two lipid monolayers having a hydrophobic "tail" region and a hydrophilic "head" region. In the membrane bilayer, the hydrophobic (nonpolar) "tails" of the lipid monolayers orient toward the center of the bilayer, whereas the hydrophilic (polar) "heads" orient toward the aqueous phase.

The liposomes of the present invention may be formed by any of the methods known in the art. Several methods may be used to form the liposomes of the present invention. For example, multilamellar vesicles (MLVs), stable plurilamellar vesicles (SPLVs), small unilamellar vesicles (SUV), or reverse phase evaporation vesicles (REV) may be used. Preferably, however, MLVs are extruded through filters forming large unilamellar vesicles (LUVs) of sizes dependent upon the filter size utilized. In general, polycarbonate filters of 30, 50, 60, 100, 200 or 800 nm pores may be used. In this method, disclosed in Cullis et al., U.S. Pat. No. 5,008,050, relevant portions of which are incorporated by reference herein, the liposome suspension may be repeatedly passed through the extrusion device resulting in a population of liposomes of homogeneous size distribution.

For example, the filtering may be performed through a straight-through membrane filter (a Nuclepore polycarbonate filter) or a tortuous path filter (e.g. a Nuclepore Membrafil filter (mixed cellulose esters) of 0.1 μ m size), or by alternative size reduction techniques such as homogenization. The size of the liposomes may vary from about 0.03 to above about 2 microns in diameter; preferably about 0.05 to 0.3 microns and most preferably about 0.1 to about 0.2 microns. The size range includes liposomes that are MLVs, SPLVs, or LUVs.

Lipids which can be used in the liposome formulations of the present invention include synthetic or natural phospholipids and may include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylinositol (PI), sphingomyelin (SPM) and cardiolipin, among others, either alone or in combination, and also in combination with cholesterol. The phospholipids useful in the present invention may also include dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG). In other embodiments, distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC), or hydrogenated soy phosphatidylcholine (HSPC) may also be used. Dimyristoylphosphatidylcholine (DMPC) and diarachidonoylphosphatidylcholine (DAPC) may similarly be used.

During preparation of the liposomes, organic solvents may also be used to suspend the lipids. Suitable organic solvents for use in the present invention include those with a variety of polarities and dielectric properties, which solubilize the lipids, for example, chloroform, methanol, ethanol, dimethylsulfoxide (DMSO), methylene chloride, and solvent mixtures such as benzene:methanol (70:30), among others. As a result, solutions (mixtures in which the lipids and other components are uniformly distributed throughout) containing the lipids are formed. Solvents are generally chosen on the basis of their biocompatibility, low toxicity, and solubilization abilities.

To encapsulate the VEGF receptor antagonist(s) of the inventions into the liposomes, the methods described in U.S. Pat. No. 5,380,531, relevant portions of which are incorporated by reference herein, may be used with the analog(s) of the present invention.

Liposomes containing the VEGF analog(s) of the present invention may be used therapeutically in mammals, especially humans, in the treatment of a number of disease states or pharmacological conditions which require sustained release formulations as well as repeated administration. The mode of administration of the liposomes containing the agents of the present invention may determine the sites and cells in the organism to which the VEGF analog may be delivered.

The liposomes of the present invention may be administered alone but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. The preparations may be injected parenterally, for example, intravenously. For parenteral administration, they can be used, for example, in the form of a sterile aqueous solution which may contain other solutes, for example, enough salts or glucose to make the solution isotonic, should isotonicity be necessary or desired. The liposomes of the present invention may also be employed subcutaneously or intramuscularly. Other uses, depending upon the particular properties of the preparation, may be envisioned by those skilled in the art.

For the oral mode of administration, the liposomal formulations of the present invention can be used in the form of tablets, capsules, lozenges, troches, powders, syrups, elixirs, aqueous solutions and suspensions, and the like. In the case of tablets, carriers which can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, lubricating agents, and talc are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents can be added.

For the topical mode of administration, the pharmaceutical formulations of the present invention may be incorporated into dosage forms such as a solution, suspension, gel, oil, ointment or salve, and the like. Preparation of such topical formulations are described in the art of pharmaceutical formulations as exemplified, for example, by Gennaro et al. (1995) *Remington's Pharmaceutical Sciences*, Mack Publishing. For topical application, the compositions could also be administered as a powder or spray, particularly in aerosol form. For administration to humans in the treatment of disease states or pharmacological conditions, the prescribing physician will ultimately determine the appropriate dosage of the agent for a given human subject, and this can be expected to vary according to the age, weight and response of the individual as well as the pharmacokinetics of the agent used.

The pharmaceutical compositions of the invention further comprise a depot formulation of biopolymers such as biodegradable microspheres. Biodegradable microspheres are used to control drug release rates and to target drugs to specific sites in the body, thereby optimizing their therapeutic response, decreasing toxic side effects, and eliminating the inconvenience of repeated injections. Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.

The biodegradable microspheres used in the context of the invention are formed with a polymer which delays the release of the proteins and maintains, at the site of action, a therapeutically effective concentration for a prolonged period of time. The polymer can be chosen from ethylcellulose, polystyrene, poly(ϵ -caprolactone), poly(lactic acid) and poly(lactic acid-co-glycolic acid) (PLGA). PLGA copolymer is one of the synthetic biodegradable and biocompatible polymers that has reproducible and slow-release characteristics. An advantage of PLGA copolymers is that their degradation rate ranges from months to years and is a function of the polymer molecular weight and the ratio of polylactic acid to polyglycolic acid residues. Several products using PLGA for parenteral applications are currently on the market, including Lupron Depot and Zoladex in the United States and Enantone Depot, Decapeptil, and Pariodel_LA in Europe (see Yonsei, Med J. 2000 December; 41(6):720-34 for review).

The pharmaceutical compositions of the invention may further be prepared, packaged, or sold in a formulation suitable for nasal administration as increased permeability has been shown through the tight junction of the nasal epithelium (Pietro and Woolley, *The Science behind Nastech's intranasal drug delivery technology*. Manufacturing Chemist, August, 2003). Such formulations may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Pharmaceutical compositions of the invention formulated for nasal delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

In some embodiments, the compositions of the invention may be administered by inhalation. For inhalation therapy, the active ingredients may be in a solution useful for administration by metered dose inhalers or in a form suitable for a dry powder inhaler. In another embodiment, the compositions are suitable for administration by bronchial lavage.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

The VEGF receptor antagonists of the present invention can be administered acutely (i.e., during the onset or shortly after events leading to inflammation), or can be administered during the course of a degenerative disease to reduce or ameliorate the progression of symptoms that would otherwise occur. The timing and interval of administration is varied according to the subject's symptoms, and can be administered at an interval of several hours to several days, over a time course of hours, days, weeks or longer, as would be determined by one skilled in the art. A typical daily regime can be from about 0.01 µg/kg body weight per day, from about 1 mg/kg body weight per day, from about 10 mg/kg body weight per day, from about 100 mg/kg body weight per day, and from about 1 g/kg body weight per day.

The VEGF receptor antagonists of the invention may be administered intravenously, orally, intranasally, intraocularly, intramuscularly, intrathecally, or by any suitable route in view of the VEGF protein, the protein formulation and the disease to be treated. Modified VEGF for the treatment of inflammatory arthritis can be injected directly into the synovial fluid. Modified VEGF for the treatment of solid tumors may be injected directly into the tumor. Modified VEGF for the treatment of skin diseases may be applied topically, for instance in the form of a lotion or spray. Intrathecal administration, i.e. for the treatment of brain tumors, can comprise injection directly in to the brain. Alternatively, modified VEGF may be coupled or conjugated to a second molecule (a "carrier"), which is a peptide or non-proteinaceous moiety selected for its ability to penetrate the blood-brain barrier and transport the active agent across the blood-brain barrier. Examples of suitable carriers are disclosed in U.S. Pat. Nos. 4,902,505; 5,604,198; and 5,017,566, which are herein incorporated by reference in their entirety.

An alternative method of administering the VEGF receptor antagonists of the present invention is carried out by administering to the subject a vector carrying a nucleic acid sequence encoding the modified VEGF protein, where the vector is capable of directing expression and secretion of the protein. Suitable vectors are typically viral vectors, including DNA viruses, RNA viruses, and retroviruses. Techniques for utilizing vector delivery systems and carrying out gene therapy are known in the art (see Lundstrom, 2003, *Trends Biotechnol.* 21(3):117-22, for a recent review).

Transgenic Animals

The production of transgenic non-human animals that contain a modified VEGF construct with increased receptor bind-

ing affinity and optionally antagonistic properties is contemplated in one embodiment of the present invention.

The successful production of transgenic, non-human animals has been described in a number of patents and publications, such as, for example U.S. Pat. No. 6,291,740 (issued Sep. 18, 2001); U.S. Pat. No. 6,281,408 (issued Aug. 28, 2001); and U.S. Pat. No. 6,271,436 (issued Aug. 7, 2001) the contents of which are hereby incorporated by reference in their entirety.

The ability to alter the genetic make-up of animals, such as domesticated mammals including cows, pigs, goats, horses, cattle, and sheep, allows a number of commercial applications. These applications include the production of animals which express large quantities of exogenous proteins in an easily harvested form (e.g., expression into the milk or blood), the production of animals with increased weight gain, feed efficiency, carcass composition, milk production or content, disease resistance and resistance to infection by specific microorganisms and the production of animals having enhanced growth rates or reproductive performance. Animals which contain exogenous DNA sequences in their genome are referred to as transgenic animals.

The most widely used method for the production of transgenic animals is the microinjection of DNA into the pronuclei of fertilized embryos (Wall et al., *J. Cell. Biochem.* 49:113 [1992]). Other methods for the production of transgenic animals include the infection of embryos with retroviruses or with retroviral vectors. Infection of both pre- and post-implantation mouse embryos with either wild-type or recombinant retroviruses has been reported (Janenich, *Proc. Natl. Acad. Sci. USA* 73:1260 [1976]; Janenich et al., *Cell* 24:519 [1981]; Stuhlmann et al., *Proc. Natl. Acad. Sci. USA* 81:7151 [1984]; Jahner et al., *Proc. Natl. Acad. Sci. USA* 82:6927 [1985]; Van der Putten et al., *Proc. Natl. Acad. Sci. USA* 82:6148-6152 [1985]; Stewart et al., *EMBO J.* 6:383-388 [1987]).

An alternative means for infecting embryos with retroviruses is the injection of virus or virus-producing cells into the blastocoele of mouse embryos (Jahner, D. et al., *Nature* 298: 623 [1982]). The introduction of transgenes into the germline of mice has been reported using intrauterine retroviral infection of the midgestation mouse embryo (Jahner et al., *supra* [1982]). Infection of bovine and ovine embryos with retroviruses or retroviral vectors to create transgenic animals has been reported. These protocols involve the microinjection of retroviral particles or growth arrested (i.e., mitomycin C-treated) cells which shed retroviral particles into the perivitelline space of fertilized eggs or early embryos (PCT International Application WO 90/08832 [1990]; and Haskell and Bowen, *Mol. Reprod. Dev.*, 40:386 [1995]. PCT International Application WO 90/08832 describes the injection of wild-type feline leukemia virus B into the perivitelline space of sheep embryos at the 2 to 8 cell stage. Fetuses derived from injected embryos were shown to contain multiple sites of integration.

U.S. Pat. No. 6,291,740 (issued Sep. 18, 2001) describes the production of transgenic animals by the introduction of exogenous DNA into pre-maturation oocytes and mature, unfertilized oocytes (i.e., pre-fertilization oocytes) using retroviral vectors which transduce dividing cells (e.g., vectors derived from murine leukemia virus [MLV]). This patent also describes methods and compositions for cytomegalovirus promoter-driven, as well as mouse mammary tumor LTR expression of various recombinant proteins.

U.S. Pat. No. 6,281,408 (issued Aug. 28, 2001) describes methods for producing transgenic animals using embryonic stem cells. Briefly, the embryonic stem cells are used in a

mixed cell co-culture with a morula to generate transgenic animals. Foreign genetic material is introduced into the embryonic stem cells prior to co-culturing by, for example, electroporation, microinjection or retroviral delivery. ES cells transfected in this manner are selected for integrations of the gene via a selection marker such as neomycin.

U.S. Pat. No. 6,271,436 (issued Aug. 7, 2001) describes the production of transgenic animals using methods including isolation of primordial germ cells, culturing these cells to produce primordial germ cell-derived cell lines, transforming both the primordial germ cells and the cultured cell lines, and using these transformed cells and cell lines to generate transgenic animals. The efficiency at which transgenic animals are generated is greatly increased, thereby allowing the use of homologous recombination in producing transgenic non-rodent animal species.

Kits Containing Modified VEGF Proteins

In a further embodiment, the present invention provides kits containing a VEGF analog and/or VEGF analog fusion proteins, which can be used, for instance, for therapeutic or non-therapeutic applications. The kit comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which includes a VEGF analog or VEGF fusion protein that is effective for therapeutic or non-therapeutic applications, such as described above. The label on the container indicates that the composition is used for a specific therapy or non-therapeutic application, and may also indicate directions for either in vivo or in vitro use, such as those described above.

The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. The kit of the invention may also include a control consisting of wild-type VEGF such as wild-type VEGF₁₆₅ or VEGF_{165b}.

The following examples are provided to describe and illustrate the present invention. As such, they should not be construed to limit the scope of the invention. Those in the art will appreciate that many other embodiments also fall within the scope of the invention, as it is described herein above and in the claims.

EXAMPLES

Example 1

Design of VEGF Receptor Antagonists

VEGF-A antagonists of the present invention were designed to increase receptor binding affinity and decrease bioactivity as compared to wild-type VEGF-A. One method by which this was done was by adding a positive charge to the loops of VEGF-A. This approach to design super-antagonists involves a combination of different methods known in the art including but not limited to homology modeling, sequence comparisons, charge scanning mutagenesis, and linking monomers and introduction of mutations in the context of linked monomers.

Vammin, or snake venom VEGF, has been shown to bind to KDR-IgG with high affinity and strongly stimulate proliferation of vascular endothelial cells in vitro (see Yamazaki et al., 2003, J. Biol. Chem. 278, 51985-51988, which is herein incorporated by reference in its entirety). VEGF-A receptor antagonists were designed based on VEGF₁₆₅ homology to

vammin. VEGF₁₆₅ has glutamate residues at positions 72 and 73, whereas vammin contains a glycine and lysine residue at these positions, respectively. By modifying VEGF-A to contain two basic amino acid residues at positions 72 and 73, the modified VEGF-A demonstrated a significant increase in receptor binding affinity compared to wild-type VEGF-A (FIG. 3A).

Example 2

Characterization of VEGF Receptor Antagonists

VEGF analogs I83K, E44R, E72RE73R, E67K and Q87K were created and assayed for their ability to bind to KDR and to decrease cell proliferation compared to wild-type VEGF.

Methods

VEGF analogs expressed by yeast cells were incubated with immobilized KDR-Fc and the ability of the analogs to bind to KDR-Fc was assayed. The binding assay was performed as follows:

1. Nunc MaxiSorp™ 96 microwell plates were coated with 150 ng/well KDR-Fc (R & D System, Inc.) and 100 µl 50 mM sodium bicarbonate buffer (15 mM Na₂CO₃+35 mM NaHCO₃) at pH 9.6. A separate plate was used for each VEGF analog and wild-type VEGF tested.

2. The plates were incubated at 4° C. overnight.

3. The next day, the wells were washed three times in washing buffer (0.05% tween in PBS).

4. The wells were blocked with PBS with 3% BSA, 0.03% tween for 1 hour at room temperature.

5. After blocking, the wells were washed three times in washing buffer (0.05% tween in PBS).

6. VEGF-A (wild-type or mutant) were added at different concentrations to the wells in 50 µl binding buffer (1% BSA and 0.03% tween in PBS).

7. ¹²⁵I-labeled VEGF-A (wild-type or mutant) at 70,000 cpm/well (PerkinElmer) was added to each well in 50 µl binding buffer (1% BSA and 0.03% tween in PBS).

8. The contents of the wells were mixed and incubated for 2 hours at room temperature with slow shaking.

9. The wells were washed three times with washing buffer (0.05% tween in PBS).

10. To each well, 120 µl of lysis buffer (0.2 M NaOH+0.5% SDS) was added. Plate was shaken vigorously for 20 minutes at room temperature.

11. The lysis buffer from each well was transferred to an individual tube. The wells were washed with lysis buffer two times additional times and combined with the lysis solution buffer in the corresponding tube.

12. The measure of binding for wild-type VEGF-A and various VEGF-A mutants was determined by counting with a gamma counter.

The ability of HUVEC endothelial cells to proliferate in the presence of the VEGF analogs was assayed as follows:

1. HUVEC endothelial cells (passage 6) were seeded into 96 well plates at 3,000 cells/well using Media-200 with growth factors and incubated overnight.

2. After overnight incubation, the media was removed and Media 199 (Invitrogen) with 2% dialysis FBS (Invitrogen) was added.

3. Cells were incubated for 20 hours.

4. Wild-type VEGF-A and VEGF-A analogs were serially diluted in Media 199 with 2% dialysis FBS in the 96-well plates, starting at 200 ng/well.

5. The media was removed from each well and replaced with 200 µl/well diluted VEGF media.

6. Cells were incubated at 37° C. for 72 hours.

35

7. Cell proliferation was analyzed using Promega's Cell-Titer-Glo® Luminescent Cell Viability Assay. Briefly, Cell-Titer buffer was thawed, transferred into CellTiter-Glo substrate, and mixed well to make substrate mixture. 100 µl growth media was removed from each well into a new 96 well plate and mixed well with 100 µl substrate mixture. The plates were shaken for 2 minutes and incubated at room temperature for an additional ten minutes.

8. Plates were read for luminescent signal using a plate reader with integration time set at 250 mS (Tecan).

Analysis

The receptor binding affinity of the I83K analog to KDR-Fc was slightly less than that of wild-type VEGF-A (FIG. 1A). However, the I83K analog demonstrated a significant decrease in endothelial cell proliferation compared to wild-type VEGF-A (FIG. 1B). VEGF-A analogs E44R, EE72/73RR, E67K and Q87K all demonstrated an increase in receptor cell binding affinity compared to wild-type VEGF-A

36

(FIGS. 2A, 3A, 4, 5 and 6). However, analogs E44R and EE72/73RR demonstrated little to no change in endothelial cell proliferation (FIGS. 2B and 3B). These results show that VEGF₁₆₅ analogs comprising I83K can effectively function as a VEGF-A receptor antagonist. Further, although VEGF-A analogs E44R and EE72/73RR were unable to decrease endothelial cell proliferation alone, when added to I83K, these modifications have the potential of further increasing receptor binding affinity.

All publications, patents and patent applications discussed in this application are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments, thereof, and may details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 73

<210> SEQ ID NO 1

<211> LENGTH: 576

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

```

atgaactttc tgctgtcttg ggtgcattgg agccttgcct tgctgetcta cctccaccat      60
gccaaagtgt cccaggctgc acccatggca gaaggaggag gccagaatca tcacgaagtg      120
gtgaagttca tggatgtcta tcagcgcagc tactgccatc caatcgagac cctggtggac      180
atcttccagg agtacacctga tgagatcgag tacatcttca agccatcctg tgtgcccctg      240
atgcgatgcg ggggctgctg caatgacgag ggccctggagt gtgtgcccac tgaggagtcc      300
aacatcacca tgcagattat gcggatcaaa cctcaccaag gccagcacat aggagagatg      360
agcttcctac agcacaacaa atgtgaatgc agaccaaaga aagatagagc aagacaagaa      420
aatccctgtg ggccttgctc agagcggaga aagcatttgt ttgtacaaga tccgcagacg      480
tgtaaatgtt cctgcaaaaa cacagactcg cgttgcaagg cgaggcagct tgagttaaac      540
gaacgtactt gcagatgtga caagccgagg cgggtga                                576

```

<210> SEQ ID NO 2

<211> LENGTH: 191

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1             5             10             15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20             25             30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35             40             45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50             55             60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65             70             75             80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85             90             95

```

-continued

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
 100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
 115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
 130 135 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
 145 150 155 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
 165 170 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 180 185 190

<210> SEQ ID NO 3
 <211> LENGTH: 26
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala
 20 25

<210> SEQ ID NO 4
 <211> LENGTH: 165
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
 1 5 10 15

Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
 20 25 30

Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys
 35 40 45

Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu
 50 55 60

Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
 65 70 75 80

Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
 85 90 95

Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
 100 105 110

Gln Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe
 115 120 125

Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser
 130 135 140

Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys
 145 150 155 160

Asp Lys Pro Arg Arg
 165

<210> SEQ ID NO 5
 <211> LENGTH: 147
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 5

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1           5           10           15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
           20           25           30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
           35           40           45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
           50           55           60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
           65           70           75           80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
           85           90           95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
           100          105          110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
           115          120          125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Cys Asp Lys
           130          135          140

Pro Arg Arg
145

```

<210> SEQ ID NO 6

<211> LENGTH: 121

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

```

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
1           5           10           15

Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
           20           25           30

Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys
           35           40           45

Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu
           50           55           60

Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
           65           70           75           80

Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
           85           90           95

Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
           100          105          110

Gln Glu Asn Cys Asp Lys Pro Arg Arg
           115          120

```

<210> SEQ ID NO 7

<211> LENGTH: 171

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1           5           10           15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
           20           25           30

```

Gly	Gly	Gln	Asn	His	His	Glu	Val	Val	Lys	Phe	Met	Asp	Val	Tyr	Gln
	35						40					45			
Arg	Ser	Tyr	Cys	His	Pro	Ile	Glu	Thr	Leu	Val	Asp	Ile	Phe	Gln	Glu
	50					55					60				
Tyr	Pro	Asp	Glu	Ile	Glu	Tyr	Ile	Phe	Lys	Pro	Ser	Cys	Val	Pro	Leu
65					70					75					80
Met	Arg	Cys	Gly	Gly	Cys	Cys	Asn	Asp	Glu	Gly	Leu	Glu	Cys	Val	Pro
				85					90					95	
Thr	Glu	Glu	Ser	Asn	Ile	Thr	Met	Gln	Ile	Met	Arg	Ile	Lys	Pro	His
			100					105					110		
Gln	Gly	Gln	His	Ile	Gly	Glu	Met	Ser	Phe	Leu	Gln	His	Asn	Lys	Cys
		115					120					125			
Glu	Cys	Arg	Pro	Lys	Lys	Asp	Arg	Ala	Arg	Gln	Glu	Lys	Lys	Ser	Val
	130					135					140				
Arg	Gly	Lys	Gly	Lys	Gly	Gln	Lys	Arg	Lys	Arg	Lys	Lys	Ser	Arg	Tyr
145					150					155					160
Lys	Ser	Trp	Ser	Val	Cys	Asp	Lys	Pro	Arg	Arg					
				165					170						

```
<210> SEQ ID NO 8
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
```

<400> SEQUENCE: 8

[illegible]

```
<210> SEQ ID NO 9
<211> LENGTH: 174
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
```

<400> SEQUENCE: 9

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30

-continued

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
 35 40 45
 Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
 50 55 60
 Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
 65 70 75 80
 Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
 85 90 95
 Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
 100 105 110
 Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
 115 120 125
 Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
 130 135 140
 Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
 145 150 155 160
 Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Met
 165 170

<210> SEQ ID NO 10
 <211> LENGTH: 148
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
 1 5 10 15
 Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
 20 25 30
 Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys
 35 40 45
 Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu
 50 55 60
 Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
 65 70 75 80
 Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
 85 90 95
 Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
 100 105 110
 Gln Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe
 115 120 125
 Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser
 130 135 140
 Arg Cys Lys Met
 145

<210> SEQ ID NO 11
 <211> LENGTH: 606
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

atgaactttc tgctgtcttg ggtgcattgg agccttgccct tgctgtctcta cctccacccat 60
 gccaaagtggc cccaggctgc acccatggca gaaggaggag ggcagaatca tcacgaagtg 120
 gtgaagtcca tggatgtcta tcagcgcagc tactgccatc caatcgagac cctgggtggac 180

-continued

atcttcagg agtacctga tgagatcgag tacatctca agccatcctg tgtgccctg	240
atgcgatgcg ggggctgctg caatgacgag ggcctggagt gtgtgccac tgaggagtcc	300
aacatcacca tgcagattat gcggatcaaa cctcaccaag gccagcacat aggagagatg	360
agcttcctac agcacaacaa atgtgaatgc agaccaaaga aagatagagc aagacaagaa	420
aatccctgtg ggccttgctc agagcggaga aagcatttgt ttgtacaaga tccgcagacg	480
tgtaaatggt cctgcaaaaa cacagactcg cgttgcaagg cgaggcagct tgagttaaac	540
gaacgtactt gcagatctct caccagggaaa gactgataca gaacgatcga tacagaaacc	600
acgctg	606

<210> SEQ ID NO 12
 <211> LENGTH: 191
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu	1	5	10	15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly	20	25	30	
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln	35	40	45	
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu	50	55	60	
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu	65	70	75	80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro	85	90	95	
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His	100	105	110	
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys	115	120	125	
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly	130	135	140	
Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr	145	150	155	160
Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln	165	170	175	
Leu Glu Leu Asn Glu Arg Thr Cys Arg Ser Leu Thr Arg Lys Asp	180	185	190	

<210> SEQ ID NO 13
 <211> LENGTH: 165
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys	1	5	10	15
Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu	20	25	30	
Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys	35	40	45	
Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu	50	55	60	

-continued

Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
65 70 75 80

Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
85 90 95

Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
100 105 110

Gln Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe
115 120 125

Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser
130 135 140

Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Ser
145 150 155 160

Leu Thr Arg Lys Asp
165

<210> SEQ ID NO 14
<211> LENGTH: 209
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Pro
145 150 155 160

Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro
165 170 175

Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala
180 185 190

Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg
195 200 205

Arg

<210> SEQ ID NO 15
<211> LENGTH: 183
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

-continued

Ala	Pro	Met	Ala	Glu	Gly	Gly	Gly	Gln	Asn	His	His	Glu	Val	Val	Lys
1				5					10					15	
Phe	Met	Asp	Val	Tyr	Gln	Arg	Ser	Tyr	Cys	His	Pro	Ile	Glu	Thr	Leu
			20					25					30		
Val	Asp	Ile	Phe	Gln	Glu	Tyr	Pro	Asp	Glu	Ile	Glu	Tyr	Ile	Phe	Lys
		35					40					45			
Pro	Ser	Cys	Val	Pro	Leu	Met	Arg	Cys	Gly	Gly	Cys	Cys	Asn	Asp	Glu
	50					55					60				
Gly	Leu	Glu	Cys	Val	Pro	Thr	Glu	Glu	Ser	Asn	Ile	Thr	Met	Gln	Ile
65					70					75					80
Met	Arg	Ile	Lys	Pro	His	Gln	Gly	Gln	His	Ile	Gly	Glu	Met	Ser	Phe
			85						90					95	
Leu	Gln	His	Asn	Lys	Cys	Glu	Cys	Arg	Pro	Lys	Lys	Asp	Arg	Ala	Arg
			100					105					110		
Gln	Glu	Lys	Lys	Ser	Val	Arg	Gly	Lys	Gly	Lys	Gly	Gln	Lys	Arg	Lys
		115					120					125			
Arg	Lys	Lys	Ser	Arg	Pro	Cys	Gly	Pro	Cys	Ser	Glu	Arg	Arg	Lys	His
	130					135					140				
Leu	Phe	Val	Gln	Asp	Pro	Gln	Thr	Cys	Lys	Cys	Ser	Cys	Lys	Asn	Thr
145					150					155					160
Asp	Ser	Arg	Cys	Lys	Ala	Arg	Gln	Leu	Glu	Leu	Asn	Glu	Arg	Thr	Cys
			165					170						175	
Arg	Cys	Asp	Lys	Pro	Arg	Arg									
			180												

<210> SEQ ID NO 16

<211> LENGTH: 215

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met	Asn	Phe	Leu	Leu	Ser	Trp	Val	His	Trp	Ser	Leu	Ala	Leu	Leu	Leu
1			5						10					15	
Tyr	Leu	His	His	Ala	Lys	Trp	Ser	Gln	Ala	Ala	Pro	Met	Ala	Glu	Gly
		20					25						30		
Gly	Gly	Gln	Asn	His	His	Glu	Val	Val	Lys	Phe	Met	Asp	Val	Tyr	Gln
		35				40						45			
Arg	Ser	Tyr	Cys	His	Pro	Ile	Glu	Thr	Leu	Val	Asp	Ile	Phe	Gln	Glu
	50				55						60				
Tyr	Pro	Asp	Glu	Ile	Glu	Tyr	Ile	Phe	Lys	Pro	Ser	Cys	Val	Pro	Leu
65				70						75					80
Met	Arg	Cys	Gly	Gly	Cys	Cys	Asn	Asp	Glu	Gly	Leu	Glu	Cys	Val	Pro
			85					90						95	
Thr	Glu	Glu	Ser	Asn	Ile	Thr	Met	Gln	Ile	Met	Arg	Ile	Lys	Pro	His
		100					105						110		
Gln	Gly	Gln	His	Ile	Gly	Glu	Met	Ser	Phe	Leu	Gln	His	Asn	Lys	Cys
		115				120						125			
Glu	Cys	Arg	Pro	Lys	Lys	Asp	Arg	Ala	Arg	Gln	Glu	Lys	Lys	Ser	Val
	130					135					140				
Arg	Gly	Lys	Gly	Lys	Gly	Gln	Lys	Arg	Lys	Arg	Lys	Lys	Ser	Arg	Tyr
145				150					155						160
Lys	Ser	Trp	Ser	Val	Pro	Cys	Gly	Pro	Cys	Ser	Glu	Arg	Arg	Lys	His
			165					170						175	
Leu	Phe	Val	Gln	Asp	Pro	Gln	Thr	Cys	Lys	Cys	Ser	Cys	Lys	Asn	Thr
			180					185						190	

-continued

Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys
 195 200 205

Arg Cys Asp Lys Pro Arg Arg
 210 215

<210> SEQ ID NO 17
 <211> LENGTH: 189
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
 1 5 10 15
 Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
 20 25 30
 Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys
 35 40 45
 Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu
 50 55 60
 Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
 65 70 75 80
 Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
 85 90 95
 Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
 100 105 110
 Gln Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys
 115 120 125
 Arg Lys Lys Ser Arg Tyr Lys Ser Trp Ser Val Pro Cys Gly Pro Cys
 130 135 140
 Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys
 145 150 155 160
 Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu
 165 170 175
 Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 180 185

<210> SEQ ID NO 18
 <211> LENGTH: 232
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1 5 10 15
 Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
 20 25 30
 Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
 35 40 45
 Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
 50 55 60
 Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
 65 70 75 80
 Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
 85 90 95
 Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
 100 105 110

-continued

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
 115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
 130 135 140

Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
 145 150 155 160

Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
 165 170 175

Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
 180 185 190

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
 195 200 205

Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
 210 215 220

Cys Arg Cys Asp Lys Pro Arg Arg
 225 230

<210> SEQ ID NO 19
 <211> LENGTH: 206
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
 1 5 10 15

Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
 20 25 30

Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys
 35 40 45

Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu
 50 55 60

Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
 65 70 75 80

Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
 85 90 95

Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
 100 105 110

Gln Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys
 115 120 125

Arg Lys Lys Ser Arg Tyr Lys Ser Trp Ser Val Tyr Val Gly Ala Arg
 130 135 140

Cys Cys Leu Met Pro Trp Ser Leu Pro Gly Pro His Pro Cys Gly Pro
 145 150 155 160

Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys
 165 170 175

Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu
 180 185 190

Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 195 200 205

<210> SEQ ID NO 20
 <211> LENGTH: 576
 <212> TYPE: DNA
 <213> ORGANISM: Macaca fascicularis

<400> SEQUENCE: 20

-continued

```

atgaactttc tgetgtcttg ggtgcattgg agccttgcc tgetgtgtga cctccaccat    60
gccaaagtggg cccagggtgc acccatggca gaaggaggag ggcagaatca tcacgaagtg    120
gtgaagtcca tggatgtcta tcagcgcagc tactgccatc caatcgagac cctgggtggac    180
atcttccagg agtacctga tgagattgag tacatcttca agccatcctg tgtgcccctg    240
atgcgatgtg ggggctgctg caatgacgag ggcctggagt gtgtgccac tgaggagtcc    300
aacatcacca tgcagattat gcggatcaaaa cctcaccaag gccagcacat aggagagatg    360
agcttccctac agcacaacaa atgtgaatgc agaccaaaga aagatagagc aagacaagaa    420
aatccctgtg ggccttgctc agagcggaga aagcatttgt ttgtacaaga tccgcagacg    480
tgtaaattgt cctgcaaaaa cacagactcg cgttgcaagg cgaggcagct tgagttaaac    540
gaacgtactt gcagatgtga caagccgagg cggtga                                576

```

```

<210> SEQ ID NO 21
<211> LENGTH: 191
<212> TYPE: PRT
<213> ORGANISM: Macaca fascicularis

```

```

<400> SEQUENCE: 21

```

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1      5      10      15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20     25     30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35     40     45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50     55     60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65     70     75     80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85     90     95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100    105    110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115    120    125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130    135    140
Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145    150    155    160
Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165    170    175
Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180    185    190

```

```

<210> SEQ ID NO 22
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Macaca fascicularis

```

```

<400> SEQUENCE: 22

```

```

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
1      5      10      15
Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
20     25     30

```

-continued

Val	Asp	Ile	Phe	Gln	Glu	Tyr	Pro	Asp	Glu	Ile	Glu	Tyr	Ile	Phe	Lys
	35						40					45			
Pro	Ser	Cys	Val	Pro	Leu	Met	Arg	Cys	Gly	Gly	Cys	Cys	Asn	Asp	Glu
	50					55					60				
Gly	Leu	Glu	Cys	Val	Pro	Thr	Glu	Glu	Ser	Asn	Ile	Thr	Met	Gln	Ile
	65				70					75				80	
Met	Arg	Ile	Lys	Pro	His	Gln	Gly	Gln	His	Ile	Gly	Glu	Met	Ser	Phe
			85					90						95	
Leu	Gln	His	Asn	Lys	Cys	Glu	Cys	Arg	Pro	Lys	Lys	Asp	Arg	Ala	Arg
			100					105					110		
Gln	Glu	Asn	Pro	Cys	Gly	Pro	Cys	Ser	Glu	Arg	Arg	Lys	His	Leu	Phe
		115				120						125			
Val	Gln	Asp	Pro	Gln	Thr	Cys	Lys	Cys	Ser	Cys	Lys	Asn	Thr	Asp	Ser
	130					135					140				
Arg	Cys	Lys	Ala	Arg	Gln	Leu	Glu	Leu	Asn	Glu	Arg	Thr	Cys	Arg	Cys
	145				150				155						160
Asp	Lys	Pro	Arg	Arg											
				165											

<210> SEQ ID NO 23
 <211> LENGTH: 573
 <212> TYPE: DNA
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 23

atgaactttc tgctctcttg ggtacattgg agccttgccct tgctgctcta ccttcacccat	60
gccaagtgggt cccaggctgc acccatggca gaaggagggc agaaacccca cgaagtgggtg	120
aagttcatgg atgtctacca gcgcagcttc tgccgtccca tcgagaccct ggtggacatc	180
ttccaggagt acccagatga gattgagttc attttcaagc cgtcctgtgt gccctgatg	240
cggtgcgggg gctgctgtaa tgacgaaagt ctggagtgtg tgcccactga ggagtccaac	300
atcaccatgc agattatgcg gatcaaacct caccaaagcc agcacatagg agagatgagc	360
ttcctacagc ataacaaatg tgaatgcaga ccaaagaaag ataaagcaag gcaagaaaat	420
ccctgtgggc cttgctcaga gcggagaaag catttgtttg tacaagatcc gcagacgtgt	480
aaatgttcct gcaaaaacac agactcgcgt tgcaaggcga ggcagcttga gttaaaccgaa	540
cgtacttgca gatgtgacaa gccgagggcg tga	573

<210> SEQ ID NO 24
 <211> LENGTH: 190
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 24

Met	Asn	Phe	Leu	Leu	Ser	Trp	Val	His	Trp	Ser	Leu	Ala	Leu	Leu	Leu
1			5						10				15		
Tyr	Leu	His	His	Ala	Lys	Trp	Ser	Gln	Ala	Ala	Pro	Met	Ala	Glu	Gly
		20					25					30			
Gly	Gln	Lys	Pro	His	Glu	Val	Val	Lys	Phe	Met	Asp	Val	Tyr	Gln	Arg
	35					40					45				
Ser	Phe	Cys	Arg	Pro	Ile	Glu	Thr	Leu	Val	Asp	Ile	Phe	Gln	Glu	Tyr
	50				55					60					
Pro	Asp	Glu	Ile	Glu	Phe	Ile	Phe	Lys	Pro	Ser	Cys	Val	Pro	Leu	Met
	65			70				75					80		
Arg	Cys	Gly	Gly	Cys	Cys	Asn	Asp	Glu	Ser	Leu	Glu	Cys	Val	Pro	Thr

	85		90		95										
Glu	Glu	Phe	Asn	Ile	Thr	Met	Gln	Ile	Met	Arg	Ile	Lys	Pro	His	Gln
	100						105					110			
Ser	Gln	His	Ile	Gly	Glu	Met	Ser	Phe	Leu	Gln	His	Asn	Lys	Cys	Glu
	115					120					125				
Cys	Arg	Pro	Lys	Lys	Asp	Lys	Ala	Arg	Gln	Glu	Asn	Pro	Cys	Gly	Pro
	130				135						140				
Cys	Ser	Glu	Arg	Arg	Lys	His	Leu	Phe	Val	Gln	Asp	Pro	Gln	Thr	Cys
	145				150					155					160
Lys	Cys	Ser	Cys	Lys	Asn	Thr	Asp	Ser	Arg	Cys	Lys	Ala	Arg	Gln	Leu
			165					170						175	
Glu	Leu	Asn	Glu	Arg	Thr	Cys	Arg	Cys	Asp	Lys	Pro	Arg	Arg		
		180					185						190		
 <210> SEQ ID NO 25 <211> LENGTH: 164 <212> TYPE: PRT <213> ORGANISM: Bos taurus <400> SEQUENCE: 25 Ala Pro Met Ala Glu Gly Gly Gln Lys Pro His Glu Val Val Lys Phe 1 5 10 15 Met Asp Val Tyr Gln Arg Ser Phe Cys Arg Pro Ile Glu Thr Leu Val 20 25 30 Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Phe Ile Phe Lys Pro 35 40 45 Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser 50 55 60 Leu Glu Cys Val Pro Thr Glu Glu Phe Asn Ile Thr Met Gln Ile Met 65 70 75 80 Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser Phe Leu 85 90 95 Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Lys Ala Arg Gln 100 105 110 Glu Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val 115 120 125 Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg 130 135 140 Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp 145 150 155 160 Lys Pro Arg Arg <210> SEQ ID NO 26 <211> LENGTH: 645 <212> TYPE: DNA <213> ORGANISM: Canis familiaris <400> SEQUENCE: 26 atgaactttc tgctctcttg ggtgcattgg agccttgcct tgctgctcta cctccaccat 60 gccaaagtggg cccaggctgc gcctatggca ggaggagagc acaaacccca cgaagtgggtg 120 aatgttcattg acgtctacca gcgcagctac tgccgtccca ttgagacctt ggtggacatc 180 ttccaggagt accctgacga gatcgagtac atcttcaagc catcctgcgt gccctgatg 240 cgggtgtgggg gctgctgtaa tgatgagggc ctagagtgcg tgcccactga ggagttaaac 300 atcaccatgc agattatgcg gatcaaacct catcaaggcc agcacatagg ggagatgagt 360															

-continued

```

ttcctgcagc atagcaaatg tgaatgcaga ccaaagaaag atagagcaag gcaagaaaaa 420
aaatcaattc gaggaaaggg gaagggggcaa aaaagaaagc gcaagaaatc ccggtataaa 480
ccctggagcg ttcctgtggt gccttgctca gagcggagaa agcatttggt tgtacaagat 540
ccgcagacgt gtaaatgttc ctgcaaaaac acagactcgc gttgcaaggc gaggcagctt 600
gagttaaacg aacgtacttg cagatgtgac aagccgaggc ggtga 645

```

```

<210> SEQ ID NO 27
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Canis familiaris

```

```

<400> SEQUENCE: 27

```

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1          5          10         15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Gly Gly
20        25        30
Glu His Lys Pro His Glu Val Val Lys Phe Met Asp Val Tyr Gln Arg
35        40        45
Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr
50        55        60
Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu Met
65        70        75        80
Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro Thr
85        90        95
Glu Glu Phe Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln
100       105       110
Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Ser Lys Cys Glu
115       120       125
Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Ile Arg
130       135       140
Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr Lys
145       150       155       160
Pro Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu
165       170       175
Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp
180       185       190
Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg
195       200       205
Cys Asp Lys Pro Arg Arg
210

```

```

<210> SEQ ID NO 28
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Canis familiaris

```

```

<400> SEQUENCE: 28

```

```

Ala Pro Met Ala Gly Gly Glu His Lys Pro His Glu Val Val Lys Phe
1          5          10         15
Met Asp Val Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val
20        25        30
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
35        40        45
Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly

```

-continued

50	55	60
Leu Glu Cys Val Pro Thr	Glu Glu Phe Asn Ile	Thr Met Gln Ile Met
65	70	75 80
Arg Ile Lys Pro His Gln Gly	Gln His Ile Gly	Glu Met Ser Phe Leu
	85	90 95
Gln His Ser Lys Cys Glu Cys	Arg Pro Lys Lys Asp	Arg Ala Arg Gln
	100	105 110
Glu Lys Lys Ser Ile Arg Gly	Lys Gly Lys Gly	Gln Lys Arg Lys Arg
	115	120 125
Lys Lys Ser Arg Tyr Lys Pro	Trp Ser Val Pro	Cys Gly Pro Cys Ser
	130	135 140
Glu Arg Arg Lys His Leu Phe	Val Gln Asp Pro	Gln Thr Cys Lys Cys
145	150	155 160
Ser Cys Lys Asn Thr Asp Ser	Arg Cys Lys Ala Arg	Gln Leu Glu Leu
	165	170 175
Asn Glu Arg Thr Cys Arg Cys	Asp Lys Pro Arg Arg	
	180	185

<210> SEQ ID NO 29

<211> LENGTH: 651

<212> TYPE: DNA

<213> ORGANISM: Gallus gallus

<400> SEQUENCE: 29

```

atgaactttc tgctcacttg gatccactgg gggctggcgg cgtgctctta tctgcagagc    60
gcggaagtgt cgaaggctgc tccggccctg ggggatgggg agcgaagcc caacgaagtt    120
atcaaattcc tggaagtcta cgaacgcagc ttctgcagga caattgagac cctgggtggac    180
attttccagg agtacctga tgaggtggag tacatatcca ggccatcctg tgtgctctg    240
atgagatgtg cgggttgtg cggcgatgag ggcctagaat gtgtccctgt ggatgtgtac    300
aacgtcaca tggagatgc aagaattaaa ccccatcaga gtcagcacat agcgcacatg    360
agcttcttac agcacagtaa atgtgactgc agaccaaaga aagatgtcaa aaataaacia    420
gaaaaaaaa caaagcgagg aaaggggaag ggtcaaaaga gaaagcgcaa gaaaggccgg    480
tacaaccac ccagctttca ctgtgagcct tgctcagaga ggagaaagca cttgtttgta    540
caagatcccc agacctgtaa atgttctctg aaattcacag actcacgttg caagtcgagg    600
cagcttgagt taaacgagcg cacttgacga tgtgaaaaac cgagacggtg a          651

```

<210> SEQ ID NO 30

<211> LENGTH: 216

<212> TYPE: PRT

<213> ORGANISM: Gallus gallus

<400> SEQUENCE: 30

Met Asn Phe Leu Leu Thr Trp	Ile His Trp Gly	Leu Ala Ala Leu Leu
1	5	10 15
Tyr Leu Gln Ser Ala Glu Leu	Ser Lys Ala Ala	Pro Ala Leu Gly Asp
	20	25 30
Gly Glu Arg Lys Pro Asn Glu	Val Ile Lys Phe	Leu Glu Val Tyr Glu
	35	40 45
Arg Ser Phe Cys Arg Thr	Ile Glu Thr Leu	Val Asp Ile Phe Gln Glu
	50	55 60
Tyr Pro Asp Glu Val Glu Tyr	Ile Phe Arg Pro	Ser Cys Val Pro Leu
65	70	75 80

-continued

Met Arg Cys Ala Gly Cys Cys Gly Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Val Asp Val Tyr Asn Val Thr Met Glu Ile Ala Arg Ile Lys Pro His
100 105 110

Gln Ser Gln His Ile Ala His Met Ser Phe Leu Gln His Ser Lys Cys
115 120 125

Asp Cys Arg Pro Lys Lys Asp Val Lys Asn Lys Gln Glu Lys Lys Ser
130 135 140

Lys Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Gly Arg
145 150 155 160

Tyr Lys Pro Pro Ser Phe His Cys Glu Pro Cys Ser Glu Arg Arg Lys
165 170 175

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Phe
180 185 190

Thr Asp Ser Arg Cys Lys Ser Arg Gln Leu Glu Leu Asn Glu Arg Thr
195 200 205

Cys Arg Cys Glu Lys Pro Arg Arg
210 215

<210> SEQ ID NO 31
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: Gallus gallus

<400> SEQUENCE: 31

Ala Pro Ala Leu Gly Asp Gly Glu Arg Lys Pro Asn Glu Val Ile Lys
1 5 10 15

Phe Leu Glu Val Tyr Glu Arg Ser Phe Cys Arg Thr Ile Glu Thr Leu
20 25 30

Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Val Glu Tyr Ile Phe Arg
35 40 45

Pro Ser Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Gly Asp Glu
50 55 60

Gly Leu Glu Cys Val Pro Val Asp Val Tyr Asn Val Thr Met Glu Ile
65 70 75 80

Ala Arg Ile Lys Pro His Gln Ser Gln His Ile Ala His Met Ser Phe
85 90 95

Leu Gln His Ser Lys Cys Asp Cys Arg Pro Lys Lys Asp Val Lys Asn
100 105 110

Lys Gln Glu Lys Lys Ser Lys Arg Gly Lys Gly Lys Gly Gln Lys Arg
115 120 125

Lys Arg Lys Lys Gly Arg Tyr Lys Pro Pro Ser Phe His Cys Glu Pro
130 135 140

Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys
145 150 155 160

Lys Cys Ser Cys Lys Phe Thr Asp Ser Arg Cys Lys Ser Arg Gln Leu
165 170 175

Glu Leu Asn Glu Arg Thr Cys Arg Cys Glu Lys Pro Arg Arg
180 185 190

<210> SEQ ID NO 32
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Equus caballus

<400> SEQUENCE: 32

-continued

```

atgaactttc tgctctcttg ggtgcattgg agccttgcc tgcgtctcta cctccaccat    60
gccaagtggc cccagggtgc acccatggca gaaggagagc ataaaaccca tgaagtgggtg    120
aagttcatgg acgtctacca gcgcagctac tgccgtccaa tcgagaccct ggtggacatc    180
ttccaggagt accccgatga gatcgagtac atcttcaagc catcctgtgt gccctgatg    240
cgggtgtggg gctgctgcaa cgacgagggc ctagagtgcg tgcccactgc ggagtccaac    300
atcaccatgc agattatgcg gatcaaacct caccaaagcc aacacatagg agagatgagt    360
ttcctacagc atagcaaatg tgaatgcaga ccaaagaaag ataaagcaag gcaagaaaat    420
ccctgtgggc cttgctcaga gcggagaaaag catttgtttg tacaagatcc gcagacgtgt    480
aatgttctct gcaaaaacac agactcgcgt tgcaaggcga ggcagcttga gttaaacgaa    540
cgtacttgca gatgtgacaa gccgaggcgg tga                                573

```

```

<210> SEQ ID NO 33
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: Equus caballus

```

```

<400> SEQUENCE: 33

```

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1             5             10            15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
          20             25             30
Glu His Lys Thr His Glu Val Val Lys Phe Met Asp Val Tyr Gln Arg
          35             40             45
Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr
          50             55             60
Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu Met
          65             70             75             80
Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro Thr
          85             90             95
Ala Glu Phe Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln
          100            105            110
Ser Gln His Ile Gly Glu Met Ser Phe Leu Gln His Ser Lys Cys Glu
          115            120            125
Cys Arg Pro Lys Lys Asp Lys Ala Arg Gln Glu Asn Pro Cys Gly Pro
          130            135            140
Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys
          145            150            155            160
Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu
          165            170            175
Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
          180            185            190

```

```

<210> SEQ ID NO 34
<211> LENGTH: 164
<212> TYPE: PRT
<213> ORGANISM: Equus caballus

```

```

<400> SEQUENCE: 34

```

```

Ala Pro Met Ala Glu Gly Glu His Lys Thr His Glu Val Val Lys Phe
 1             5             10            15
Met Asp Val Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val
          20             25             30
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro

```

-continued

35	40	45
Ser Cys Val Pro Leu Met	Arg Cys Gly Gly Cys	Cys Asn Asp Glu Gly
50	55	60
Leu Glu Cys Val Pro Thr	Ala Glu Phe Asn Ile	Thr Met Gln Ile Met
65	70	75 80
Arg Ile Lys Pro His Gln	Ser Gln His Ile Gly	Glu Met Ser Phe Leu
	85	90 95
Gln His Ser Lys Cys Glu	Cys Arg Pro Lys Lys	Asp Lys Ala Arg Gln
	100	105 110
Glu Asn Pro Cys Gly Pro	Cys Ser Glu Arg Arg	Lys His Leu Phe Val
	115	120 125
Gln Asp Pro Gln Thr Cys	Lys Cys Ser Cys Lys	Asn Thr Asp Ser Arg
	130	135 140
Cys Lys Ala Arg Gln Leu	Glu Leu Asn Glu Arg	Thr Cys Arg Cys Asp
145	150	155 160
Lys Pro Arg Arg		

<210> SEQ ID NO 35
 <211> LENGTH: 573
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

```

atgaactttc tgctgtcttg ggtgcactgg accctggcct tactgctgta cctccaccat    60
gccaagtggg cccagggtgc acccaccgaca gaaggagagc agaagtccca tgaagtgatc    120
aagttcatgg acgtctacca gcgaagctac tgccgtccaa ttgagaccct ggtggacatc    180
ttccaggagt accccgacga gatagagtac atcttcaagc cgctcctgtgt gccgctgatg    240
cgctgtgcag gctgctgtaa cgatgaagcc ctggagtgcg tgcccacgct agagagcaac    300
atcaccatgc agatcatgcg gatcaaacct caccaaagcc agcacatagg agagatgagc    360
ttcctacagc acagccgatg tgaatgcaga ccaaagaaag acaggacaaa gccagaaaat    420
cactgtgagc cttgttcaga gcggagaaaag catttgtttg tccaagatcc gcagacgtgt    480
aaatgttcct gcaaaaaacac agactcgcgt tgcaaggcga ggcagcttga gttaaacgaa    540
cgtacttgca gatgtgacaa gccgaggcgg tga                                     573

```

<210> SEQ ID NO 36
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 36

Met Asn Phe Leu Leu Ser	Trp Val His Trp Thr	Leu Ala Leu Leu Leu
1	5	10 15
Tyr Leu His His Ala Lys	Trp Ser Gln Ala Ala	Pro Thr Thr Glu Gly
	20	25 30
Glu Gln Lys Ser His Glu	Val Ile Lys Phe Met	Asp Val Tyr Gln Arg
	35	40 45
Ser Tyr Cys Arg Pro Ile	Glu Thr Leu Val Asp	Ile Phe Gln Glu Tyr
	50	55 60
Pro Asp Glu Ile Glu Tyr	Ile Phe Lys Pro Ser	Cys Val Pro Leu Met
65	70	75 80
Arg Cys Ala Gly Cys Cys	Asn Asp Glu Ala Leu	Glu Cys Val Pro Thr
	85	90 95

-continued

Ser Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln
100 105 110

Ser Gln His Ile Gly Glu Met Ser Phe Leu Gln His Ser Arg Cys Glu
115 120 125

Cys Arg Pro Lys Lys Asp Arg Thr Lys Pro Glu Lys Lys Ser Val Arg
130 135 140

Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys
145 150 155 160

Ser Trp Ser Val His Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu
165 170 175

Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp
180 185 190

Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg
195 200 205

Cys Asp Lys Pro Arg Arg
210

<210> SEQ ID NO 37
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Ala Pro Thr Thr Glu Gly Glu Gln Lys Ser His Glu Val Ile Lys Phe
1 5 10 15

Met Asp Val Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val
20 25 30

Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
35 40 45

Ser Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
50 55 60

Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Ile Thr Met Gln Ile Met
65 70 75 80

Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser Phe Leu
85 90 95

Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg Thr Lys Pro
100 105 110

Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg
115 120 125

Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His Cys Glu Pro Cys Ser
130 135 140

Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys
145 150 155 160

Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu
165 170 175

Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185

<210> SEQ ID NO 38
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Sus scrofa

<400> SEQUENCE: 38

atgaactttc tgctgtcttg ggtgcattgg agccttgccct tgctgtctcta cctccaccat 60
gccaaagtggc cccaggctgc acccatggca gaaggagacc agaaacccca cgaagtggcg 120

-continued

```

aagttcatgg acgtctacca ggcagctac tgccgtccaa tcgagaccct ggtggacatc 180
ttccaggagt accccgatga gatcgagtac atcttcaagc cgtcctgtgt gccctgatg 240
cgggtgcgggg gctgctgcaa cgacgaaggt ctggagtggt tgcccactga ggagtccaac 300
atcaccatgc agattatgcg gatcaaacct caccaaggcc agcacatagg agagatgagc 360
ttcctacagc acaacaaatg tgaatgcaga ccaaagaaag atagagcgag gcaagaaaat 420
ccctgtgggc cttgctcaga gcggagaaaag catttggttg tacaagatcc gcagacgtgt 480
aatgttcct gcaaaaacac agactcgctg tgcaaggcga ggcagcttga gttaaacgaa 540
cgtacttgca gatgtgacaa gccgaggcgg tga 573

```

```

<210> SEQ ID NO 39
<211> LENGTH: 190
<212> TYPE: PRT
<213> ORGANISM: Sus scrofa

```

```

<400> SEQUENCE: 39

```

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1             5             10             15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
          20             25             30
Asp Gln Lys Pro His Glu Val Val Lys Phe Met Asp Val Tyr Gln Arg
          35             40             45
Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr
          50             55             60
Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu Met
          65             70             75             80
Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro Thr
          85             90             95
Glu Glu Phe Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln
          100            105            110
Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys Glu
          115            120            125
Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly Pro
          130            135            140
Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys
          145            150            155            160
Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu
          165            170            175
Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
          180            185            190

```

```

<210> SEQ ID NO 40
<211> LENGTH: 164
<212> TYPE: PRT
<213> ORGANISM: Sus scrofa

```

```

<400> SEQUENCE: 40

```

```

Ala Pro Met Ala Glu Gly Asp Gln Lys Pro His Glu Val Val Lys Phe
 1             5             10             15
Met Asp Val Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val
          20             25             30
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
          35             40             45
Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly

```

-continued

50	55	60
Leu Glu Cys Val Pro Thr	Glu Glu Phe Asn Ile	Thr Met Gln Ile Met
65	70	75
80		
Arg Ile Lys Pro His Gln Gly	Gln His Ile Gly	Glu Met Ser Phe Leu
85	90	95
Gln His Asn Lys Cys Glu Cys	Arg Pro Lys Lys Asp	Arg Ala Arg Gln
100	105	110
Glu Asn Pro Cys Gly Pro Cys	Ser Glu Arg Arg Lys	His Leu Phe Val
115	120	125
Gln Asp Pro Gln Thr Cys Lys	Cys Ser Cys Lys Asn	Thr Asp Ser Arg
130	135	140
Cys Lys Ala Arg Gln Leu Glu	Leu Asn Glu Arg Thr	Cys Arg Cys Asp
145	150	155
160		
Lys Pro Arg Arg		

<210> SEQ ID NO 41
 <211> LENGTH: 573
 <212> TYPE: DNA
 <213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 41

atgaactttc tgctctcttg ggtgcactgg accctggctt tactgctgta cctccaccat	60
gccaagtggg cccagggtgc acccacgaca gaaggggagc agaaagccca tgaagtgggtg	120
aagttcatgg acgtctacca gcgcagctat tgccgtccga ttgagaccct ggtggacatc	180
ttccaggagt accccgatga gatagagtat atcttcaagc cgtcctgtgt gccctaata	240
cgggtgtgagg gctgctgcaa tgatgaagcc ctggagtgcg tgcccacgct ggagagcaac	300
gtcactatgc agatcatgag gatcaaacct caccaaagcc agcacatagg agagatgagc	360
ttcctgcagc atagcagatg tgaatgcaga ccaaagaaag atagaacaaa gccagaaaat	420
cactgtgagc cttgttcaga gcggagaaaag catttgtttg tccaagatcc gcagacgtgt	480
aaatgttctt gcaaaaacac agactcgcgt tgcaaggcga ggcagcttga gttaaacgaa	540
cgtacttgca gatgtgacaa gccaaaggcg tga	573

<210> SEQ ID NO 42
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 42

Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu Leu	
1	15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr Glu Gly	
20	30
Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val Tyr Gln Arg	
35	45
Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr	
50	60
Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu Met	
65	80
Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala Leu Glu Cys Val Pro Thr	
85	95
Ser Glu Ser Asn Val Thr Met Gln Ile Met Arg Ile Lys Pro His Gln	
100	110

-continued

Ser Gln His Ile Gly Glu Met Ser Phe Leu Gln His Ser Arg Cys Glu
115 120 125

Cys Arg Pro Lys Lys Asp Arg Thr Lys Pro Glu Lys Lys Ser Val Arg
130 135 140

Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys
145 150 155 160

Ser Trp Ser Val His Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu
165 170 175

Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp
180 185 190

Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg
195 200 205

Cys Asp Lys Pro Arg Arg
210

<210> SEQ ID NO 43
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 43

Ala Pro Thr Thr Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe
1 5 10 15

Met Asp Val Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val
20 25 30

Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro
35 40 45

Ser Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
50 55 60

Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile Met
65 70 75 80

Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser Phe Leu
85 90 95

Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg Thr Lys Pro
100 105 110

Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg
115 120 125

Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His Cys Glu Pro Cys Ser
130 135 140

Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys
145 150 155 160

Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu
165 170 175

Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185

<210> SEQ ID NO 44
<211> LENGTH: 441
<212> TYPE: DNA
<213> ORGANISM: Ovis aries

<400> SEQUENCE: 44

atgaactttc tgctctcttg ggtgcattgg agccttgccct tgctgctcta ccttcacccat 60

gccaaagtggc cccaggctgc acccatggca gaaggagggc agaaacccca tgaagtgatg 120

aagttcatgg atgtctacca ggcagcttc tgccgtccca ttgagaccct ggtggacatc 180

-continued

```

ttccaggagt acccagatga gattgagttc attttcaagc cgtccctgtgt gccctgatg 240
cggtgcgggg gctgctgtaa tgacgaaagt ctggagtggtg tgcccactga ggagttcaac 300
atcaccatgc agattatgcg gatcaaacct caccaaagcc agcacatagg agagatgagt 360
ttcttacagc ataacaaatg tgaatgcaga ccaaagaaag ataaagcaag gcaagaaaaa 420
tgtgacaagc cgaggcggtg a 441

```

```

<210> SEQ ID NO 45
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Ovis aries

```

```

<400> SEQUENCE: 45

```

```

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1          5          10          15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
          20          25          30
Gly Gln Lys Pro His Glu Val Met Lys Phe Met Asp Val Tyr Gln Arg
          35          40          45
Ser Phe Cys Arg Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr
          50          55          60
Pro Asp Glu Ile Glu Phe Ile Phe Lys Pro Ser Cys Val Pro Leu Met
          65          70          75          80
Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser Leu Glu Cys Val Pro Thr
          85          90          95
Glu Glu Phe Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln
          100          105          110
Ser Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys Glu
          115          120          125
Cys Arg Pro Lys Lys Asp Lys Ala Arg Gln Glu Lys Cys Asp Lys Pro
          130          135          140
Arg Arg
145

```

```

<210> SEQ ID NO 46
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Ovis aries

```

```

<400> SEQUENCE: 46

```

```

Ala Pro Met Ala Glu Gly Gly Gln Lys Pro His Glu Val Met Lys Phe
1          5          10          15
Met Asp Val Tyr Gln Arg Ser Phe Cys Arg Pro Ile Glu Thr Leu Val
          20          25          30
Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Phe Ile Phe Lys Pro
          35          40          45
Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Ser
          50          55          60
Leu Glu Cys Val Pro Thr Glu Glu Phe Asn Ile Thr Met Gln Ile Met
          65          70          75          80
Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser Phe Leu
          85          90          95
Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Lys Ala Arg Gln
          100          105          110
Glu Lys Cys Asp Lys Pro Arg Arg
          115          120

```

-continued

<210> SEQ ID NO 47
 <211> LENGTH: 188
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

```

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1          5          10          15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
          20          25          30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
          35          40          45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
          50          55          60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
          65          70          75          80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
          85          90          95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
          100          105          110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
          115          120          125

Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
          130          135          140

Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
          145          150          155          160

Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
          165          170          175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
          180          185

```

<210> SEQ ID NO 48
 <211> LENGTH: 167
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

```

Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln Arg Lys Val Val Ser
1          5          10          15

Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val
          20          25          30

Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala Lys Gln Leu Val
          35          40          45

Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp
          50          55          60

Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile
          65          70          75          80

Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu
          85          90          95

Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Asp Ser Ala Val
          100          105          110

Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg Cys Thr Gln His His
          115          120          125

Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Ser
          130          135          140

```

-continued

Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys
145 150 155 160

Arg Cys Arg Lys Leu Arg Arg
165

<210> SEQ ID NO 49
<211> LENGTH: 207
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
20 25 30

Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
35 40 45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
50 55 60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65 70 75 80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
85 90 95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
100 105 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
115 120 125

Lys Asp Ser Ala Val Lys Pro Asp Arg Ala Ala Thr Pro His His Arg
130 135 140

Pro Gln Pro Arg Ser Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro
145 150 155 160

Ser Pro Ala Asp Ile Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala
165 170 175

His Ala Ala Pro Ser Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala
180 185 190

Ala Ala Ala Asp Ala Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
195 200 205

<210> SEQ ID NO 50
<211> LENGTH: 186
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln Arg Lys Val Val Ser
1 5 10 15

Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln Pro Arg Glu Val Val
20 25 30

Val Pro Leu Thr Val Glu Leu Met Gly Thr Val Ala Lys Gln Leu Val
35 40 45

Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys Pro Asp Asp
50 55 60

Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met Gln Ile
65 70 75 80

Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu Glu
85 90 95

-continued

Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Asp Ser Ala Val
100 105 110

Lys Pro Asp Arg Ala Ala Thr Pro His His Arg Pro Gln Pro Arg Ser
115 120 125

Val Pro Gly Trp Asp Ser Ala Pro Gly Ala Pro Ser Pro Ala Asp Ile
130 135 140

Thr His Pro Thr Pro Ala Pro Gly Pro Ser Ala His Ala Ala Pro Ser
145 150 155 160

Thr Thr Ser Ala Leu Thr Pro Gly Pro Ala Ala Ala Ala Ala Asp Ala
165 170 175

Ala Ala Ser Ser Val Ala Lys Gly Gly Ala
180 185

<210> SEQ ID NO 51
<211> LENGTH: 419
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1 5 10 15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
100 105 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
115 120 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
130 135 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
145 150 155 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
165 170 175

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
180 185 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
195 200 205

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
210 215 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225 230 235 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
245 250 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
260 265 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu

-continued

275	280	285
Glu Thr Cys Gln Cys Val	Cys Arg Ala Gly Leu Arg	Pro Ala Ser Cys
290	295	300
Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys		
305	310	315 320
Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu		
325	330	335
Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro		
340	345	350
Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys		
355	360	365
Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr		
370	375	380
Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser		
385	390	395 400
Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro		
405	410	415
Gln Met Ser		
<210> SEQ ID NO 52		
<211> LENGTH: 354		
<212> TYPE: PRT		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 52		
Met Tyr Arg Glu Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val		
1	5	10 15
Gln Leu Val Gln Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser		
20	25	30
Ser Gln Ser Thr Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser		
35	40	45
Ser Leu Glu Glu Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu		
50	55	60
Trp Arg Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg		
65	70	75 80
Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile		
85	90	95
Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser		
100	105	110
Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr		
115	120	125
Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly		
130	135	140
Cys Cys Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr		
145	150	155 160
Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro		
165	170	175
Glu Leu Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu		
180	185	190
Pro Thr Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln		
195	200	205
Ile Pro Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile		
210	215	220
Asp Met Leu Trp Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu		

-continued

225	230	235	240
Asn Pro Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala			
	245	250	255
Leu Cys Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val			
	260	265	270
Cys Lys Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys			
	275	280	285
Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His			
	290	295	300
Lys Leu Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe			
	305	310	315
His Thr Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys			
	325	330	335
Arg Phe Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys			
	340	345	350

Asn Pro

<210> SEQ ID NO 53

<211> LENGTH: 149

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly			
1	5	10	15
Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly			
	20	25	30
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly			
	35	40	45
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu			
	50	55	60
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu			
	65	70	75
Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro			
	85	90	95
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly			
	100	105	110
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys			
	115	120	125
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp			
	130	135	140
Ala Val Pro Arg Arg			
145			

<210> SEQ ID NO 54

<211> LENGTH: 131

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly Asn Gly			
1	5	10	15
Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly Arg Ser			
	20	25	30
Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu Tyr Pro			
	35	40	45

-continued

Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg
50 55 60

Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
65 70 75 80

Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly Asp Arg
85 90 95

Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys Glu Cys
100 105 110

Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp Ala Val
115 120 125

Pro Arg Arg
130

<210> SEQ ID NO 55
<211> LENGTH: 170
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1 5 10 15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
20 25 30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
35 40 45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50 55 60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65 70 75 80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85 90 95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100 105 110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115 120 125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
130 135 140

Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys
145 150 155 160

His Leu Cys Gly Asp Ala Val Pro Arg Arg
165 170

<210> SEQ ID NO 56
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly Asn Gly
1 5 10 15

Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly Arg Ser
20 25 30

Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu Tyr Pro
35 40 45

Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg
50 55 60

-continued

Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
 65 70 75 80
 Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly Asp Arg
 85 90 95
 Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys Glu Cys
 100 105 110
 Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly
 115 120 125
 Arg Gly Lys Arg Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys His Leu
 130 135 140
 Cys Gly Asp Ala Val Pro Arg Arg
 145 150

<210> SEQ ID NO 57
 <211> LENGTH: 221
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
 1 5 10 15
 Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
 20 25 30
 Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
 35 40 45
 Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
 50 55 60
 Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
 65 70 75 80
 Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
 85 90 95
 Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
 100 105 110
 Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
 115 120 125
 Glu Cys Arg His Ser Pro Gly Arg Gln Ser Pro Asp Met Pro Gly Asp
 130 135 140
 Phe Arg Ala Asp Ala Pro Ser Phe Leu Pro Pro Arg Arg Ser Leu Pro
 145 150 155 160
 Met Leu Phe Arg Met Glu Trp Gly Cys Ala Leu Thr Gly Ser Gln Ser
 165 170 175
 Ala Val Trp Pro Ser Ser Pro Val Pro Glu Glu Ile Pro Arg Met His
 180 185 190
 Pro Gly Arg Asn Gly Lys Lys Gln Gln Arg Lys Pro Leu Arg Glu Lys
 195 200 205
 Met Lys Pro Glu Arg Cys Gly Asp Ala Val Pro Arg Arg
 210 215 220

<210> SEQ ID NO 58
 <211> LENGTH: 203
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly Asn Gly
 1 5 10 15

-continued

Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly Arg Ser
 20 25 30
 Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu Tyr Pro
 35 40 45
 Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg
 50 55 60
 Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
 65 70 75 80
 Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly Asp Arg
 85 90 95
 Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys Glu Cys
 100 105 110
 Arg His Ser Pro Gly Arg Gln Ser Pro Asp Met Pro Gly Asp Phe Arg
 115 120 125
 Ala Asp Ala Pro Ser Phe Leu Pro Pro Arg Arg Ser Leu Pro Met Leu
 130 135 140
 Phe Arg Met Glu Trp Gly Cys Ala Leu Thr Gly Ser Gln Ser Ala Val
 145 150 155 160
 Trp Pro Ser Ser Pro Val Pro Glu Glu Ile Pro Arg Met His Pro Gly
 165 170 175
 Arg Asn Gly Lys Lys Gln Gln Arg Lys Pro Leu Arg Glu Lys Met Lys
 180 185 190
 Pro Glu Arg Cys Gly Asp Ala Val Pro Arg Arg
 195 200

<210> SEQ ID NO 59

<211> LENGTH: 345

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

Met Ser Leu Phe Gly Leu Leu Leu Leu Thr Ser Ala Leu Ala Gly Gln
 1 5 10 15
 Arg Gln Gly Thr Gln Ala Glu Ser Asn Leu Ser Ser Lys Phe Gln Phe
 20 25 30
 Ser Ser Asn Lys Glu Gln Asn Gly Val Gln Asp Pro Gln His Glu Arg
 35 40 45
 Ile Ile Thr Val Ser Thr Asn Gly Ser Ile His Ser Pro Arg Phe Pro
 50 55 60
 His Thr Tyr Pro Arg Asn Thr Val Leu Val Trp Arg Leu Val Ala Val
 65 70 75 80
 Glu Glu Asn Val Trp Ile Gln Leu Thr Phe Asp Glu Arg Phe Gly Leu
 85 90 95
 Glu Asp Pro Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu Val Glu
 100 105 110
 Glu Pro Ser Asp Gly Thr Ile Leu Gly Arg Trp Cys Gly Ser Gly Thr
 115 120 125
 Val Pro Gly Lys Gln Ile Ser Lys Gly Asn Gln Ile Arg Ile Arg Phe
 130 135 140
 Val Ser Asp Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile His Tyr
 145 150 155 160
 Asn Ile Val Met Pro Gln Phe Thr Glu Ala Val Ser Pro Ser Val Leu
 165 170 175
 Pro Pro Ser Ala Leu Pro Leu Asp Leu Leu Asn Asn Ala Ile Thr Ala

```

      180              185              190
Phe Ser Thr Leu Glu Asp Leu Ile Arg Tyr Leu Glu Pro Glu Arg Trp
      195              200              205

Gln Leu Asp Leu Glu Asp Leu Tyr Arg Pro Thr Trp Gln Leu Leu Gly
      210              215              220

Lys Ala Phe Val Phe Gly Arg Lys Ser Arg Val Val Asp Leu Asn Leu
      225              230              235              240

Leu Thr Glu Glu Val Arg Leu Tyr Ser Cys Thr Pro Arg Asn Phe Ser
      245              250              255

Val Ser Ile Arg Glu Glu Leu Lys Arg Thr Asp Thr Ile Phe Trp Pro
      260              265              270

Gly Cys Leu Leu Val Lys Arg Cys Gly Gly Asn Cys Ala Cys Cys Leu
      275              280              285

His Asn Cys Asn Glu Cys Gln Cys Val Pro Ser Lys Val Thr Lys Lys
      290              295              300

Tyr His Glu Val Leu Gln Leu Arg Pro Lys Thr Gly Val Arg Gly Leu
      305              310              315              320

His Lys Ser Leu Thr Asp Val Ala Leu Glu His His Glu Glu Cys Asp
      325              330              335

Cys Val Cys Arg Gly Ser Thr Gly Gly
      340              345

<210> SEQ ID NO 60
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 60

Thr Gln Ala Glu Ser Asn Leu Ser Ser Lys Phe Gln Phe Ser Ser Asn
1      5      10      15

Lys Glu Gln Asn Gly Val Gln Asp Pro Gln His Glu Arg Ile Ile Thr
20      25      30

Val Ser Thr Asn Gly Ser Ile His Ser Pro Arg Phe Pro His Thr Tyr
35      40      45

Pro Arg Asn Thr Val Leu Val Trp Arg Leu Val Ala Val Glu Glu Asn
50      55      60

Val Trp Ile Gln Leu Thr Phe Asp Glu Arg Phe Gly Leu Glu Asp Pro
65      70      75      80

Glu Asp Asp Ile Cys Lys Tyr Asp Phe Val Glu Val Glu Glu Pro Ser
85      90      95

Asp Gly Thr Ile Leu Gly Arg Trp Cys Gly Ser Gly Thr Val Pro Gly
100     105     110

Lys Gln Ile Ser Lys Gly Asn Gln Ile Arg Ile Arg Phe Val Ser Asp
115     120     125

Glu Tyr Phe Pro Ser Glu Pro Gly Phe Cys Ile His Tyr Asn Ile Val
130     135     140

Met Pro Gln Phe Thr Glu Ala Val Ser Pro Ser Val Leu Pro Pro Ser
145     150     155     160

Ala Leu Pro Leu Asp Leu Leu Asn Asn Ala Ile Thr Ala Phe Ser Thr
165     170     175

Leu Glu Asp Leu Ile Arg Tyr Leu Glu Pro Glu Arg Trp Gln Leu Asp
180     185     190

Leu Glu Asp Leu Tyr Arg Pro Thr Trp Gln Leu Leu Gly Lys Ala Phe
195     200     205

```

-continued

Val Phe Gly Arg Lys Ser Arg Val Val Asp Leu Asn Leu Leu Thr Glu
 210 215 220

Glu Val Arg Leu Tyr Ser Cys Thr Pro Arg Asn Phe Ser Val Ser Ile
 225 230 235 240

Arg Glu Glu Leu Lys Arg Thr Asp Thr Ile Phe Trp Pro Gly Cys Leu
 245 250 255

Leu Val Lys Arg Cys Gly Gly Asn Cys Ala Cys Cys Leu His Asn Cys
 260 265 270

Asn Glu Cys Gln Cys Val Pro Ser Lys Val Thr Lys Lys Tyr His Glu
 275 280 285

Val Leu Gln Leu Arg Pro Lys Thr Gly Val Arg Gly Leu His Lys Ser
 290 295 300

Leu Thr Asp Val Ala Leu Glu His His Glu Glu Cys Asp Cys Val Cys
 305 310 315 320

Arg Gly Ser Thr Gly Gly
 325

<210> SEQ ID NO 61
 <211> LENGTH: 147
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
 20 25 30

Gly Gly Gln Asn His His Glu Val Lys Phe Met Asp Val Tyr Gln
 35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
 50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
 65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
 85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
 100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
 115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Cys Asp Lys
 130 135 140

Pro Arg Arg
 145

<210> SEQ ID NO 62
 <211> LENGTH: 121
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Ala Pro Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys
 1 5 10 15

Phe Met Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu
 20 25 30

Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys
 35 40 45

-continued

```

Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu
 50                      55                      60

Gly Leu Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile
65                      70                      75                      80

Met Arg Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe
                        85                      90                      95

Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg
                100                      105                      110

Gln Glu Lys Cys Asp Lys Pro Arg Arg
    115                      120

```

```

<210> SEQ ID NO 63
<211> LENGTH: 211
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 63

```

```

Met Arg Thr Leu Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
 1                      5                      10                      15

His Val Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Val Ile Glu Arg
                20                      25                      30

Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
                35                      40                      45

Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg
    50                      55                      60

Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu
65                      70                      75                      80

Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys
                85                      90                      95

Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
    100                      105                      110

Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
    115                      120                      125

Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
    130                      135                      140

Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145                      150                      155                      160

Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
                165                      170                      175

Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp
    180                      185                      190

Thr Gly Arg Pro Arg Glu Ser Gly Lys Lys Arg Lys Arg Lys Arg Leu
    195                      200                      205

Lys Pro Thr
    210

```

```

<210> SEQ ID NO 64
<211> LENGTH: 196
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 64

```

```

Met Arg Thr Leu Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
 1                      5                      10                      15

His Val Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Val Ile Glu Arg
    20                      25                      30

```

-continued

Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
 35 40 45
 Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg
 50 55 60
 Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu
 65 70 75 80
 Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys
 85 90 95
 Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
 100 105 110
 Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
 115 120 125
 Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
 130 135 140
 Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
 145 150 155 160
 Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
 165 170 175
 Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp
 180 185 190
 Thr Asp Val Arg
 195

<210> SEQ ID NO 65
 <211> LENGTH: 1338
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1 5 10 15
 Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
 20 25 30
 Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
 35 40 45
 Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
 50 55 60
 Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
 65 70 75 80
 Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
 85 90 95
 Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
 100 105 110
 Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
 115 120 125
 Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
 130 135 140
 Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
 145 150 155 160
 Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
 165 170 175
 Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
 180 185 190
 Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
 195 200 205

-continued

Ala Thr Val Asn Gly His	Leu Tyr Lys Thr Asn Tyr	Leu Thr His Arg
210	215	220
Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val		
225	230	235 240
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr		
	245	250 255
Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys		
	260	265 270
Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His		
	275	280 285
Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys		
	290	295 300
Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys		
305	310	315 320
Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val		
	325	330 335
Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser		
	340	345 350
Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val		
	355	360 365
Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu		
	370	375 380
Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala		
385	390	395 400
Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys		
	405	410 415
Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu		
	420	425 430
Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser		
	435	440 445
Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile		
	450	455 460
Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys		
465	470	475 480
Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser		
	485	490 495
Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile		
	500	505 510
Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg		
	515	520 525
Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val		
	530	535 540
Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His		
545	550	555 560
Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser		
	565	570 575
Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu		
	580	585 590
Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys		
	595	600 605
Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met		
	610	615 620

-continued

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn	625	630	635	640
Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg	645	650	655	
Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val	660	665	670	
Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val Pro	675	680	685	
Glu Pro Gln Ile Thr Trp Phe Lys Asn Asn His Lys Ile Gln Gln Glu	690	695	700	
Pro Gly Ile Ile Leu Gly Pro Gly Ser Ser Thr Leu Phe Ile Glu Arg	705	710	715	720
Val Thr Glu Glu Asp Glu Gly Val Tyr His Cys Lys Ala Thr Asn Gln	725	730	735	
Lys Gly Ser Val Glu Ser Ser Ala Tyr Leu Thr Val Gln Gly Thr Ser	740	745	750	
Asp Lys Ser Asn Leu Glu Leu Ile Thr Leu Thr Cys Thr Cys Val Ala	755	760	765	
Ala Thr Leu Phe Trp Leu Leu Leu Thr Leu Leu Ile Arg Lys Met Lys	770	775	780	
Arg Ser Ser Ser Glu Ile Lys Thr Asp Tyr Leu Ser Ile Ile Met Asp	785	790	795	800
Pro Asp Glu Val Pro Leu Asp Glu Gln Cys Glu Arg Leu Pro Tyr Asp	805	810	815	
Ala Ser Lys Trp Glu Phe Ala Arg Glu Arg Leu Lys Leu Gly Lys Ser	820	825	830	
Leu Gly Arg Gly Ala Phe Gly Lys Val Val Gln Ala Ser Ala Phe Gly	835	840	845	
Ile Lys Lys Ser Pro Thr Cys Arg Thr Val Ala Val Lys Met Leu Lys	850	855	860	
Glu Gly Ala Thr Ala Ser Glu Tyr Lys Ala Leu Met Thr Glu Leu Lys	865	870	875	880
Ile Leu Thr His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly	885	890	895	
Ala Cys Thr Lys Gln Gly Gly Pro Leu Met Val Ile Val Glu Tyr Cys	900	905	910	
Lys Tyr Gly Asn Leu Ser Asn Tyr Leu Lys Ser Lys Arg Asp Leu Phe	915	920	925	
Phe Leu Asn Lys Asp Ala Ala Leu His Met Glu Pro Lys Lys Glu Lys	930	935	940	
Met Glu Pro Gly Leu Glu Gln Gly Lys Lys Pro Arg Leu Asp Ser Val	945	950	955	960
Thr Ser Ser Glu Ser Phe Ala Ser Ser Gly Phe Gln Glu Asp Lys Ser	965	970	975	
Leu Ser Asp Val Glu Glu Glu Glu Asp Ser Asp Gly Phe Tyr Lys Glu	980	985	990	
Pro Ile Thr Met Glu Asp Leu Ile Ser Tyr Ser Phe Gln Val Ala Arg	995	1000	1005	
Gly Met Glu Phe Leu Ser Ser Arg Lys Cys Ile His Arg Asp Leu	1010	1015	1020	
Ala Ala Arg Asn Ile Leu Leu Ser Glu Asn Asn Val Val Lys Ile	1025	1030	1035	
Cys Asp Phe Gly Leu Ala Arg Asp Ile Tyr Lys Asn Pro Asp Tyr				

1040	1045	1050
Val Arg Lys Gly Asp Thr Arg Leu Pro Leu Lys Trp Met Ala Pro 1055 1060 1065		
Glu Ser Ile Phe Asp Lys Ile Tyr Ser Thr Lys Ser Asp Val Trp 1070 1075 1080		
Ser Tyr Gly Val Leu Leu Trp Glu Ile Phe Ser Leu Gly Gly Ser 1085 1090 1095		
Pro Tyr Pro Gly Val Gln Met Asp Glu Asp Phe Cys Ser Arg Leu 1100 1105 1110		
Arg Glu Gly Met Arg Met Arg Ala Pro Glu Tyr Ser Thr Pro Glu 1115 1120 1125		
Ile Tyr Gln Ile Met Leu Asp Cys Trp His Arg Asp Pro Lys Glu 1130 1135 1140		
Arg Pro Arg Phe Ala Glu Leu Val Glu Lys Leu Gly Asp Leu Leu 1145 1150 1155		
Gln Ala Asn Val Gln Gln Asp Gly Lys Asp Tyr Ile Pro Ile Asn 1160 1165 1170		
Ala Ile Leu Thr Gly Asn Ser Gly Phe Thr Tyr Ser Thr Pro Ala 1175 1180 1185		
Phe Ser Glu Asp Phe Phe Lys Glu Ser Ile Ser Ala Pro Lys Phe 1190 1195 1200		
Asn Ser Gly Ser Ser Asp Asp Val Arg Tyr Val Asn Ala Phe Lys 1205 1210 1215		
Phe Met Ser Leu Glu Arg Ile Lys Thr Phe Glu Glu Leu Leu Pro 1220 1225 1230		
Asn Ala Thr Ser Met Phe Asp Asp Tyr Gln Gly Asp Ser Ser Thr 1235 1240 1245		
Leu Leu Ala Ser Pro Met Leu Lys Arg Phe Thr Trp Thr Asp Ser 1250 1255 1260		
Lys Pro Lys Ala Ser Leu Lys Ile Asp Leu Arg Val Thr Ser Lys 1265 1270 1275		
Ser Lys Glu Ser Gly Leu Ser Asp Val Ser Arg Pro Ser Phe Cys 1280 1285 1290		
His Ser Ser Cys Gly His Val Ser Glu Gly Lys Arg Arg Phe Thr 1295 1300 1305		
Tyr Asp His Ala Glu Leu Glu Arg Lys Ile Ala Cys Cys Ser Pro 1310 1315 1320		
Pro Pro Asp Tyr Asn Ser Val Val Leu Tyr Ser Thr Pro Pro Ile 1325 1330 1335		
<210> SEQ ID NO 66		
<211> LENGTH: 1356		
<212> TYPE: PRT		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 66		
Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu 1 5 10 15		
Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro 20 25 30		
Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr 35 40 45		
Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro 50 55 60		

Asn 65	Asn	Gln	Ser	Gly	Ser 70	Glu	Gln	Arg	Val	Glu 75	Val	Thr	Glu	Cys	Ser 80
Asp	Gly	Leu	Phe	Cys 85	Lys	Thr	Leu	Thr	Ile 90	Pro	Lys	Val	Ile	Gly 95	Asn
Asp	Thr	Gly	Ala	Tyr 100	Lys	Cys	Phe	Tyr 105	Arg	Glu	Thr	Asp	Leu 110	Ala	Ser
Val	Ile	Tyr	Val	Tyr 115	Val	Gln	Asp 120	Tyr	Arg	Ser	Pro	Phe 125	Ile	Ala	Ser
Val	Ser	Asp	Gln	His	Gly	Val 135	Val	Tyr	Ile	Thr	Glu 140	Asn	Lys	Asn	Lys
Thr 145	Val	Val	Ile	Pro	Cys 150	Leu	Gly	Ser	Ile	Ser	Asn	Leu	Asn	Val	Ser 160
Leu	Cys	Ala	Arg	Tyr 165	Pro	Glu	Lys	Arg	Phe 170	Val	Pro	Asp	Gly	Asn	Arg
Ile	Ser	Trp	Asp 180	Ser	Lys	Lys	Gly	Phe 185	Thr	Ile	Pro	Ser	Tyr 190	Met	Ile
Ser	Tyr	Ala 195	Gly	Met	Val	Phe	Cys 200	Glu	Ala	Lys	Ile	Asn 205	Asp	Glu	Ser
Tyr	Gln 210	Ser	Ile	Met	Tyr	Ile 215	Val	Val	Val	Val	Gly 220	Tyr	Arg	Ile	Tyr
Asp 225	Val	Val	Leu	Ser	Pro 230	Ser	His	Gly	Ile	Glu 235	Leu	Ser	Val	Gly	Glu 240
Lys	Leu	Val	Leu	Asn 245	Cys	Thr	Ala	Arg	Thr 250	Glu	Leu	Asn	Val	Gly 255	Ile
Asp	Phe	Asn 260	Trp	Glu	Tyr	Pro	Ser	Ser 265	Lys	His	Gln	His	Lys 270	Lys	Leu
Val	Asn 275	Arg	Asp	Leu	Lys	Thr	Gln 280	Ser	Gly	Ser	Glu	Met 285	Lys	Lys	Phe
Leu	Ser 290	Thr	Leu	Thr	Ile	Asp 295	Gly	Val	Thr	Arg	Ser 300	Asp	Gln	Gly	Leu
Tyr 305	Thr	Cys	Ala	Ala	Ser 310	Ser	Gly	Leu	Met	Thr 315	Lys	Lys	Asn	Ser	Thr 320
Phe	Val	Arg	Val	His 325	Glu	Lys	Pro	Phe 330	Val	Ala	Phe	Gly	Ser	Gly 335	Met
Glu	Ser	Leu 340	Val	Glu	Ala	Thr	Val	Gly 345	Glu	Arg	Val	Arg	Ile 350	Pro	Ala
Lys	Tyr 355	Leu	Gly	Tyr	Pro	Pro	Pro 360	Glu	Ile	Lys	Trp	Tyr 365	Lys	Asn	Gly
Ile	Pro 370	Leu	Glu	Ser	Asn 375	His	Thr	Ile	Lys	Ala	Gly 380	His	Val	Leu	Thr
Ile 385	Met	Glu	Val	Ser	Glu 390	Arg	Asp	Thr	Gly	Asn 395	Tyr	Thr	Val	Ile	Leu 400
Thr	Asn	Pro	Ile	Ser 405	Lys	Glu	Lys	Gln	Ser 410	His	Val	Val	Ser	Leu 415	Val
Val	Tyr	Val 420	Pro	Pro	Gln	Ile	Gly	Glu 425	Lys	Ser	Leu	Ile	Ser 430	Pro	Val
Asp	Ser	Tyr 435	Gln	Tyr	Gly	Thr	Thr 440	Gln	Thr	Leu	Thr	Cys 445	Thr	Val	Tyr
Ala	Ile 450	Pro	Pro	Pro	His 455	His	Ile	His	Trp	Tyr	Trp	Gln 460	Leu	Glu	Glu
Glu 465	Cys	Ala	Asn	Glu	Pro 470	Ser	Gln	Ala	Val	Ser 475	Val	Thr	Asn	Pro	Tyr 480
Pro	Cys	Glu	Glu	Trp	Arg	Ser	Val	Glu	Asp	Phe	Gln	Gly	Gly	Asn	Lys

-continued

485								490					495				
Ile	Glu	Val	Asn 500	Lys	Asn	Gln	Phe	Ala 505	Leu	Ile	Glu	Gly	Lys 510	Asn	Lys		
Thr	Val	Ser	Thr 515	Leu	Val	Ile	Gln 520	Ala	Ala	Asn	Val	Ser 525	Ala	Leu	Tyr		
Lys	Cys	Glu	Ala 530	Val	Asn	Lys 535	Val	Gly	Arg	Gly	Glu 540	Arg	Val	Ile	Ser		
Phe 545	His	Val	Thr	Arg	Gly 550	Pro	Glu	Ile	Thr	Leu 555	Gln	Pro	Asp	Met	Gln 560		
Pro	Thr	Glu	Gln 565	Glu	Ser	Val	Ser	Leu	Trp 570	Cys	Thr	Ala	Asp	Arg 575	Ser		
Thr	Phe	Glu	Asn 580	Leu	Thr	Trp	Tyr	Lys 585	Leu	Gly	Pro	Gln	Pro 590	Leu	Pro		
Ile	His	Val	Gly 595	Glu	Leu	Pro	Thr 600	Pro	Val	Cys	Lys	Asn 605	Leu	Asp	Thr		
Leu	Trp 610	Lys	Leu	Asn	Ala	Thr 615	Met	Phe	Ser	Asn	Ser 620	Thr	Asn	Asp	Ile		
Leu 625	Ile	Met	Glu	Leu	Lys 630	Asn	Ala	Ser	Leu	Gln 635	Asp	Gln	Gly	Asp	Tyr 640		
Val	Cys	Leu	Ala 645	Gln	Asp	Arg	Lys	Thr	Lys 650	Lys	Arg	His	Cys	Val 655	Val		
Arg	Gln	Leu	Thr 660	Val	Leu	Glu	Arg	Val 665	Ala	Pro	Thr	Ile	Thr 670	Gly	Asn		
Leu	Glu	Asn 675	Gln	Thr	Thr	Ser	Ile 680	Gly	Glu	Ser	Ile	Glu 685	Val	Ser	Cys		
Thr	Ala 690	Ser	Gly	Asn	Pro	Pro 695	Pro	Gln	Ile	Met	Trp 700	Phe	Lys	Asp	Asn		
Glu 705	Thr	Leu	Val	Glu	Asp 710	Ser	Gly	Ile	Val	Leu 715	Lys	Asp	Gly	Asn	Arg 720		
Asn	Leu	Thr	Ile 725	Arg	Arg	Val	Arg	Lys	Glu 730	Asp	Glu	Gly	Leu	Tyr 735	Thr		
Cys	Gln	Ala	Cys 740	Ser	Val	Leu	Gly	Cys 745	Ala	Lys	Val	Glu	Ala 750	Phe	Phe		
Ile	Ile	Glu	Gly 755	Ala	Gln	Glu	Lys 760	Thr	Asn	Leu	Glu	Ile 765	Ile	Ile	Leu		
Val	Gly 770	Thr	Ala	Val	Ile 775	Ala	Met	Phe	Phe	Trp 780	Leu	Leu	Leu	Val	Ile		
Ile 785	Leu	Arg	Thr	Val	Lys 790	Arg	Ala	Asn	Gly	Gly 795	Glu	Leu	Lys	Thr	Gly 800		
Tyr	Leu	Ser	Ile 805	Val	Met	Asp	Pro	Asp	Glu 810	Leu	Pro	Leu	Asp	Glu	His 815		
Cys	Glu	Arg	Leu 820	Pro	Tyr	Asp	Ala	Ser 825	Lys	Trp	Glu	Phe 830	Pro	Arg	Asp		
Arg	Leu	Lys 835	Leu	Gly	Lys	Pro	Leu 840	Gly	Arg	Gly	Ala	Phe 845	Gly	Gln	Val		
Ile 850	Glu	Ala	Asp	Ala	Phe	Gly 855	Ile	Asp	Lys	Thr	Ala 860	Thr	Cys	Arg	Thr		
Val 865	Ala	Val	Lys	Met	Leu 870	Lys	Glu	Gly	Ala	Thr 875	His	Ser	Glu	His	Arg 880		
Ala	Leu	Met	Ser 885	Glu	Leu	Lys	Ile	Leu	Ile 890	His	Ile	Gly	His	His	Leu		
Asn	Val	Val	Asn 900	Leu	Leu	Gly	Ala	Cys 905	Thr	Lys	Pro	Gly 910	Gly	Pro	Leu		

-continued

Met Val Ile Val Glu Phe Cys Lys Phe Gly Asn Leu Ser Thr Tyr Leu		
915	920	925
Arg Ser Lys Arg Asn Glu Phe Val Pro Tyr Lys Thr Lys Gly Ala Arg		
930	935	940
Phe Arg Gln Gly Lys Asp Tyr Val Gly Ala Ile Pro Val Asp Leu Lys		
945	950	955 960
Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly		
	965 970	975
Phe Val Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Pro		
	980 985	990
Glu Asp Leu Tyr Lys Asp Phe Leu Thr Leu Glu His Leu Ile Cys Tyr		
	995 1000	1005
Ser Phe Gln Val Ala Lys Gly Met Glu Phe Leu Ala Ser Arg Lys		
1010	1015	1020
Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu Ser Glu		
1025	1030	1035
Lys Asn Val Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile		
1040	1045	1050
Tyr Lys Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg Leu Pro		
1055	1060	1065
Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp Arg Val Tyr Thr		
1070	1075	1080
Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile		
1085	1090	1095
Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu		
1100	1105	1110
Glu Phe Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro		
1115	1120	1125
Asp Tyr Thr Thr Pro Glu Met Tyr Gln Thr Met Leu Asp Cys Trp		
1130	1135	1140
His Gly Glu Pro Ser Gln Arg Pro Thr Phe Ser Glu Leu Val Glu		
1145	1150	1155
His Leu Gly Asn Leu Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys		
1160	1165	1170
Asp Tyr Ile Val Leu Pro Ile Ser Glu Thr Leu Ser Met Glu Glu		
1175	1180	1185
Asp Ser Gly Leu Ser Leu Pro Thr Ser Pro Val Ser Cys Met Glu		
1190	1195	1200
Glu Glu Glu Val Cys Asp Pro Lys Phe His Tyr Asp Asn Thr Ala		
1205	1210	1215
Gly Ile Ser Gln Tyr Leu Gln Asn Ser Lys Arg Lys Ser Arg Pro		
1220	1225	1230
Val Ser Val Lys Thr Phe Glu Asp Ile Pro Leu Glu Glu Pro Glu		
1235	1240	1245
Val Lys Val Ile Pro Asp Asp Asn Gln Thr Asp Ser Gly Met Val		
1250	1255	1260
Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp Arg Thr Lys Leu		
1265	1270	1275
Ser Pro Ser Phe Gly Gly Met Val Pro Ser Lys Ser Arg Glu Ser		
1280	1285	1290
Val Ala Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln Ser Gly		
1295	1300	1305

-continued

Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr Ser Ser Glu Glu
 1310 1315 1320
 Ala Glu Leu Leu Lys Leu Ile Glu Ile Gly Val Gln Thr Gly Ser
 1325 1330 1335
 Thr Ala Gln Ile Leu Gln Pro Asp Ser Gly Thr Thr Leu Ser Ser
 1340 1345 1350
 Pro Pro Val
 1355

<210> SEQ ID NO 67
 <211> LENGTH: 1298
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Met Gln Arg Gly Ala Ala Leu Cys Leu Arg Leu Trp Leu Cys Leu Gly
 1 5 10 15
 Leu Leu Asp Gly Leu Val Ser Gly Tyr Ser Met Thr Pro Pro Thr Leu
 20 25 30
 Asn Ile Thr Glu Glu Ser His Val Ile Asp Thr Gly Asp Ser Leu Ser
 35 40 45
 Ile Ser Cys Arg Gly Gln His Pro Leu Glu Trp Ala Trp Pro Gly Ala
 50 55 60
 Gln Glu Ala Pro Ala Thr Gly Asp Lys Asp Ser Glu Asp Thr Gly Val
 65 70 75 80
 Val Arg Asp Cys Glu Gly Thr Asp Ala Arg Pro Tyr Cys Lys Val Leu
 85 90 95
 Leu Leu His Glu Val His Ala Asn Asp Thr Gly Ser Tyr Val Cys Tyr
 100 105 110
 Tyr Lys Tyr Ile Lys Ala Arg Ile Glu Gly Thr Thr Ala Ala Ser Ser
 115 120 125
 Tyr Val Phe Val Arg Asp Phe Glu Gln Pro Phe Ile Asn Lys Pro Asp
 130 135 140
 Thr Leu Leu Val Asn Arg Lys Asp Ala Met Trp Val Pro Cys Leu Val
 145 150 155 160
 Ser Ile Pro Gly Leu Asn Val Thr Leu Arg Ser Gln Ser Ser Val Leu
 165 170 175
 Trp Pro Asp Gly Gln Glu Val Val Trp Asp Asp Arg Arg Gly Met Leu
 180 185 190
 Val Ser Thr Pro Leu Leu His Asp Ala Leu Tyr Leu Gln Cys Glu Thr
 195 200 205
 Thr Trp Gly Asp Gln Asp Phe Leu Ser Asn Pro Phe Leu Val His Ile
 210 215 220
 Thr Gly Asn Glu Leu Tyr Asp Ile Gln Leu Leu Pro Arg Lys Ser Leu
 225 230 235 240
 Glu Leu Leu Val Gly Glu Lys Leu Val Leu Asn Cys Thr Val Trp Ala
 245 250 255
 Glu Phe Asn Ser Gly Val Thr Phe Asp Trp Asp Tyr Pro Gly Lys Gln
 260 265 270
 Ala Glu Arg Gly Lys Trp Val Pro Glu Arg Arg Ser Gln Gln Thr His
 275 280 285
 Thr Glu Leu Ser Ser Ile Leu Thr Ile His Asn Val Ser Gln His Asp
 290 295 300
 Leu Gly Ser Tyr Val Cys Lys Ala Asn Asn Gly Ile Gln Arg Phe Arg
 305 310 315 320

Glu	Ser	Thr	Glu	Val	Ile	Val	His	Glu	Asn	Pro	Phe	Ile	Ser	Val	Glu	
				325					330					335		
Trp	Leu	Lys	Gly	Pro	Ile	Leu	Glu	Ala	Thr	Ala	Gly	Asp	Glu	Leu	Val	
			340					345					350			
Lys	Leu	Pro	Val	Lys	Leu	Ala	Ala	Tyr	Pro	Pro	Pro	Glu	Phe	Gln	Trp	
		355					360					365				
Tyr	Lys	Asp	Gly	Lys	Ala	Leu	Ser	Gly	Arg	His	Ser	Pro	His	Ala	Leu	
	370					375				380						
Val	Leu	Lys	Glu	Val	Thr	Glu	Ala	Ser	Thr	Gly	Thr	Tyr	Thr	Leu	Ala	
385					390					395					400	
Leu	Trp	Asn	Ser	Ala	Ala	Gly	Leu	Arg	Arg	Asn	Ile	Ser	Leu	Glu	Leu	
				405					410					415		
Val	Val	Asn	Val	Pro	Pro	Gln	Ile	His	Glu	Lys	Glu	Ala	Ser	Ser	Pro	
			420					425					430			
Ser	Ile	Tyr	Ser	Arg	His	Ser	Arg	Gln	Ala	Leu	Thr	Cys	Thr	Ala	Tyr	
		435					440					445				
Gly	Val	Pro	Leu	Pro	Leu	Ser	Ile	Gln	Trp	His	Trp	Arg	Pro	Trp	Thr	
	450					455					460					
Pro	Cys	Lys	Met	Phe	Ala	Gln	Arg	Ser	Leu	Arg	Arg	Arg	Gln	Gln	Gln	
465					470					475					480	
Asp	Leu	Met	Pro	Gln	Cys	Arg	Asp	Trp	Arg	Ala	Val	Thr	Thr	Gln	Asp	
				485					490					495		
Ala	Val	Asn	Pro	Ile	Glu	Ser	Leu	Asp	Thr	Trp	Thr	Glu	Phe	Val	Glu	
			500					505					510			
Gly	Lys	Asn	Lys	Thr	Val	Ser	Lys	Leu	Val	Ile	Gln	Asn	Ala	Asn	Val	
		515					520					525				
Ser	Ala	Met	Tyr	Lys	Cys	Val	Val	Ser	Asn	Lys	Val	Gly	Gln	Asp	Glu	
	530					535					540					
Arg	Leu	Ile	Tyr	Phe	Tyr	Val	Thr	Thr	Ile	Pro	Asp	Gly	Phe	Thr	Ile	
545					550					555					560	
Glu	Ser	Lys	Pro	Ser	Glu	Glu	Leu	Leu	Glu	Gly	Gln	Pro	Val	Leu	Leu	
				565					570					575		
Ser	Cys	Gln	Ala	Asp	Ser	Tyr	Lys	Tyr	Glu	His	Leu	Arg	Trp	Tyr	Arg	
			580					585					590			
Leu	Asn	Leu	Ser	Thr	Leu	His	Asp	Ala	His	Gly	Asn	Pro	Leu	Leu	Leu	
			595				600					605				
Asp	Cys	Lys	Asn	Val	His	Leu	Phe	Ala	Thr	Pro	Leu	Ala	Ala	Ser	Leu	
	610					615					620					
Glu	Glu	Val	Ala	Pro	Gly	Ala	Arg	His	Ala	Thr	Leu	Ser	Leu	Ser	Ile	
625					630					635					640	
Pro	Arg	Val	Ala	Pro	Glu	His	Glu	Gly	His	Tyr	Val	Cys	Glu	Val	Gln	
			645						650					655		
Asp	Arg	Arg	Ser	His												

Arg	Val	Arg	Glu	Glu	Asp	Ala	Gly	Arg	Tyr	Leu	Cys	Ser	Val	Cys	Asn
			740					745					750		
Ala	Lys	Gly	Cys	Val	Asn	Ser	Ser	Ala	Ser	Val	Ala	Val	Glu	Gly	Ser
		755					760				765				
Glu	Asp	Lys	Gly	Ser	Met	Glu	Ile	Val	Ile	Leu	Val	Gly	Thr	Gly	Val
		770				775					780				
Ile	Ala	Val	Phe	Phe	Trp	Val	Leu	Leu	Leu	Leu	Ile	Phe	Cys	Asn	Met
785					790					795				800	
Arg	Arg	Pro	Ala	His	Ala	Asp	Ile	Lys	Thr	Gly	Tyr	Leu	Ser	Ile	Ile
			805						810					815	
Met	Asp	Pro	Gly	Glu	Val	Pro	Leu	Glu	Glu	Gln	Cys	Glu	Tyr	Leu	Ser
		820						825					830		
Tyr	Asp	Ala	Ser	Gln	Trp	Glu	Phe	Pro	Arg	Glu	Arg	Leu	His	Leu	Gly
		835					840					845			
Arg	Val	Leu	Gly	Tyr	Gly	Ala	Phe	Gly	Lys	Val	Val	Glu	Ala	Ser	Ala
		850				855					860				
Phe	Gly	Ile	His	Lys	Gly	Ser	Ser	Cys	Asp	Thr	Val	Ala	Val	Lys	Met
865					870					875				880	
Leu	Lys	Glu	Gly	Ala	Thr	Ala	Ser	Glu	His	Arg	Ala	Leu	Met	Ser	Glu
			885						890					895	
Leu	Lys	Ile	Leu	Ile	His	Ile	Gly	Asn	His	Leu	Asn	Val	Val	Asn	Leu
		900						905					910		
Leu	Gly	Ala	Cys	Thr	Lys	Pro	Gln	Gly	Pro	Leu	Met	Val	Ile	Val	Glu
		915					920					925			
Phe	Cys	Lys	Tyr	Gly	Asn	Leu	Ser	Asn	Phe	Leu	Arg	Ala	Lys	Arg	Asp
	930					935					940				
Ala	Phe	Ser	Pro	Cys	Ala	Glu	Lys	Ser	Pro	Glu	Gln	Arg	Gly	Arg	Phe
945					950					955					960
Arg	Ala	Met	Val	Glu	Leu	Ala	Arg	Leu	Asp	Arg	Arg	Arg	Pro	Gly	Ser
			965						970					975	
Ser	Asp	Arg	Val	Leu	Phe	Ala	Arg	Phe	Ser	Lys	Thr	Glu	Gly	Gly	Ala
		980						985					990		
Arg	Arg	Ala	Ser	Pro	Asp	Gln	Glu	Ala	Glu	Asp	Leu	Trp	Leu	Ser	Pro
		995					1000					1005			
Leu	Thr	Met	Glu	Asp	Leu	Val	Cys	Tyr	Ser	Phe	Gln	Val	Ala	Arg	
	1010					1015					1020				
Gly	Met	Glu	Phe	Leu	Ala	Ser	Arg	Lys	Cys	Ile	His	Arg	Asp	Leu	
	1025					1030					1035				
Ala	Ala	Arg	Asn	Ile	Leu	Leu	Ser	Glu	Ser	Asp	Val	Val	Lys	Ile	
	1040					1045					1050				
Cys	Asp	Phe	Gly	Leu	Ala	Arg	Asp	Ile	Tyr	Lys	Asp	Pro	Asp	Tyr	
	1055					1060					1065				
Val	Arg	Lys	Gly	Ser	Ala	Arg	Leu	Pro	Leu	Lys	Trp	Met	Ala	Pro	
	1070					1075					1080				
Glu	Ser	I													

-continued

1145	1150	1155
Arg Pro Ala Phe Ser Glu Leu Val Glu Ile Leu Gly Asp Leu Leu		
1160	1165	1170
Gln Gly Arg Gly Leu Gln Glu Glu Glu Glu Val Cys Met Ala Pro		
1175	1180	1185
Arg Ser Ser Gln Ser Ser Glu Glu Gly Ser Phe Ser Gln Val Ser		
1190	1195	1200
Thr Met Ala Leu His Ile Ala Gln Ala Asp Ala Glu Asp Ser Pro		
1205	1210	1215
Pro Ser Leu Gln Arg His Ser Leu Ala Ala Arg Tyr Tyr Asn Trp		
1220	1225	1230
Val Ser Phe Pro Gly Cys Leu Ala Arg Gly Ala Glu Thr Arg Gly		
1235	1240	1245
Ser Ser Arg Met Lys Thr Phe Glu Glu Phe Pro Met Thr Pro Thr		
1250	1255	1260
Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp Ser Gly Met Val		
1265	1270	1275
Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg His Arg Gln		
1280	1285	1290
Glu Ser Gly Phe Arg		
1295		

<210> SEQ ID NO 68

<211> LENGTH: 923

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Met Glu Arg Gly Leu Pro Leu Leu Cys Ala Val Leu Ala Leu Val Leu		
1	5	10 15
Ala Pro Ala Gly Ala Phe Arg Asn Asp Lys Cys Gly Asp Thr Ile Lys		
	20	25 30
Ile Glu Ser Pro Gly Tyr Leu Thr Ser Pro Gly Tyr Pro His Ser Tyr		
	35	40 45
His Pro Ser Glu Lys Cys Glu Trp Leu Ile Gln Ala Pro Asp Pro Tyr		
	50	55 60
Gln Arg Ile Met Ile Asn Phe Asn Pro His Phe Asp Leu Glu Asp Arg		
	65	70 75 80
Asp Cys Lys Tyr Asp Tyr Val Glu Val Phe Asp Gly Glu Asn Glu Asn		
	85	90 95
Gly His Phe Arg Gly Lys Phe Cys Gly Lys Ile Ala Pro Pro Pro Val		
	100	105 110
Val Ser Ser Gly Pro Phe Leu Phe Ile Lys Phe Val Ser Asp Tyr Glu		
	115	120 125
Thr His Gly Ala Gly Phe Ser Ile Arg Tyr Glu Ile Phe Lys Arg Gly		
	130	135 140
Pro Glu Cys Ser Gln Asn Tyr Thr Thr Pro Ser Gly Val Ile Lys Ser		
	145	150 155 160
Pro Gly Phe Pro Glu Lys Tyr Pro Asn Ser Leu Glu Cys Thr Tyr Ile		
	165	170 175
Val Phe Ala Pro Lys Met Ser Glu Ile Ile Leu Glu Phe Glu Ser Phe		
	180	185 190
Asp Leu Glu Pro Asp Ser Asn Pro Pro Gly Gly Met Phe Cys Arg Tyr		
	195	200 205

-continued

Asp	Arg	Leu	Glu	Ile	Trp	Asp	Gly	Phe	Pro	Asp	Val	Gly	Pro	His	Ile
210						215					220				
Gly	Arg	Tyr	Cys	Gly	Gln	Lys	Thr	Pro	Gly	Arg	Ile	Arg	Ser	Ser	Ser
225					230					235					240
Gly	Ile	Leu	Ser	Met	Val	Phe	Tyr	Thr	Asp	Ser	Ala	Ile	Ala	Lys	Glu
				245					250					255	
Gly	Phe	Ser	Ala	Asn	Tyr	Ser	Val	Leu	Gln	Ser	Ser	Val	Ser	Glu	Asp
			260					265					270		
Phe	Lys	Cys	Met	Glu	Ala	Leu	Gly	Met	Glu	Ser	Gly	Glu	Ile	His	Ser
		275					280					285			
Asp	Gln	Ile	Thr	Ala	Ser	Ser	Gln	Tyr	Ser	Thr	Asn	Trp	Ser	Ala	Glu
290						295					300				
Arg	Ser	Arg	Leu	Asn	Tyr	Pro	Glu	Asn	Gly	Trp	Thr	Pro	Gly	Glu	Asp
305					310					315					320
Ser	Tyr	Arg	Glu	Trp	Ile	Gln	Val	Asp	Leu	Gly	Leu	Leu	Arg	Phe	Val
				325					330					335	
Thr	Ala	Val	Gly	Thr	Gln	Gly	Ala	Ile	Ser	Lys	Glu	Thr	Lys	Lys	Lys
			340					345					350		
Tyr	Tyr	Val	Lys	Thr	Tyr	Lys	Ile	Asp	Val	Ser	Ser	Asn	Gly	Glu	Asp
		355					360					365			
Trp	Ile	Thr	Ile	Lys	Glu	Gly	Asn	Lys	Pro	Val	Leu	Phe	Gln	Gly	Asn
370						375					380				
Thr	Asn	Pro	Thr	Asp	Val	Val	Val	Ala	Val	Phe	Pro	Lys	Pro	Leu	Ile
385					390					395					400
Thr	Arg	Phe	Val	Arg	Ile	Lys	Pro	Ala	Thr	Trp	Glu	Thr	Gly	Ile	Ser
				405					410					415	
Met	Arg	Phe	Glu	Val	Tyr	Gly	Cys	Lys	Ile	Thr	Asp	Tyr	Pro	Cys	Ser
			420					425					430		
Gly	Met	Leu	Gly	Met	Val	Ser	Gly	Leu	Ile	Ser	Asp	Ser	Gln	Ile	Thr
		435					440					445			
Ser	Ser	Asn	Gln	Gly	Asp	Arg	Asn	Trp	Met	Pro	Glu	Asn	Ile	Arg	Leu
450						455					460				
Val	Thr	Ser	Arg	Ser	Gly	Trp	Ala	Leu	Pro	Pro	Ala	Pro	His	Ser	Tyr
465					470					475					480
Ile	Asn	Glu	Trp	Leu	Gln	Ile	Asp	Leu	Gly	Glu	Glu	Lys	Ile	Val	Arg
				485					490					495	
Gly	Ile	Ile	Ile	Gln	Gly	Gly	Lys	His	Arg	Glu	Asn	Lys	Val	Phe	Met
			500					505					510		
Arg	Lys	Phe	Lys	Ile	Gly	Tyr	Ser	Asn	Asn	Gly	Ser	Asp	Trp	Lys	Met
		515					520					525			
Ile	Met	Asp	Asp	Ser	Lys	Arg	Lys	Ala	Lys	Ser	Phe	Glu	Gly	Asn	Asn
530						535					540				
Asn	Tyr	Asp	Thr	Pro	Glu	Leu	Arg	Thr	Phe	Pro	Ala	Leu	Ser	Thr	Arg
545					550					555					560
Phe	Ile	Arg	Ile	Tyr	Pro	Glu	Arg	Ala	Thr	His	Gly	Gly	Leu	Gly	Leu
				565				570						575	
Arg	Met	Glu	Leu	Leu	Gly	Cys	Glu	Val	Glu	Ala	Pro	Thr	Ala	Gly	Pro
			580					585					590		
Thr	Thr	Pro	Asn	Gly	Asn	Leu	Val	Asp	Glu	Cys	Asp	Asp	Asp	Gln	Ala
		595				600						605			
Asn	Cys	His	Ser	Gly	Thr	Gly	Asp	Asp	Phe	Gln	Leu	Thr	Gly	Gly	Thr
610					615						620				
Thr	Val	Leu	Ala	Thr	Glu	Lys	Pro	Thr	Val	Ile	Asp	Ser	Thr	Ile	Gln

-continued

625	630	635	640
Ser Glu Phe Pro Thr Tyr Gly Phe Asn Cys Glu Phe Gly Trp Gly Ser			
	645	650	655
His Lys Thr Phe Cys His Trp Glu His Asp Asn His Val Gln Leu Lys			
	660	665	670
Trp Ser Val Leu Thr Ser Lys Thr Gly Pro Ile Gln Asp His Thr Gly			
	675	680	685
Asp Gly Asn Phe Ile Tyr Ser Gln Ala Asp Glu Asn Gln Lys Gly Lys			
	690	695	700
Val Ala Arg Leu Val Ser Pro Val Val Tyr Ser Gln Asn Ser Ala His			
	705	710	715
Cys Met Thr Phe Trp Tyr His Met Ser Gly Ser His Val Gly Thr Leu			
	725	730	735
Arg Val Lys Leu Arg Tyr Gln Lys Pro Glu Glu Tyr Asp Gln Leu Val			
	740	745	750
Trp Met Ala Ile Gly His Gln Gly Asp His Trp Lys Glu Gly Arg Val			
	755	760	765
Leu Leu His Lys Ser Leu Lys Leu Tyr Gln Val Ile Phe Glu Gly Glu			
	770	775	780
Ile Gly Lys Gly Asn Leu Gly Gly Ile Ala Val Asp Asp Ile Ser Ile			
	785	790	795
Asn Asn His Ile Ser Gln Glu Asp Cys Ala Lys Pro Ala Asp Leu Asp			
	805	810	815
Lys Lys Asn Pro Glu Ile Lys Ile Asp Glu Thr Gly Ser Thr Pro Gly			
	820	825	830
Tyr Glu Gly Glu Gly Glu Gly Asp Lys Asn Ile Ser Arg Lys Pro Gly			
	835	840	845
Asn Val Leu Lys Thr Leu Glu Pro Ile Leu Ile Thr Ile Ile Ala Met			
	850	855	860
Ser Ala Leu Gly Val Leu Leu Gly Ala Val Cys Gly Val Val Leu Tyr			
	865	870	875
Cys Ala Cys Trp His Asn Gly Met Ser Glu Arg Asn Leu Ser Ala Leu			
	885	890	895
Glu Asn Tyr Asn Phe Glu Leu Val Asp Gly Val Lys Leu Lys Lys Asp			
	900	905	910
Lys Leu Asn Thr Gln Ser Thr Tyr Ser Glu Ala			
	915	920	

<210> SEQ ID NO 69
 <211> LENGTH: 931
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Met Asp Met Phe Pro Leu Thr Trp Val Phe Leu Ala Leu Tyr Phe Ser			
1	5	10	15
Arg His Gln Val Arg Gly Gln Pro Asp Pro Pro Cys Gly Gly Arg Leu			
	20	25	30
Asn Ser Lys Asp Ala Gly Tyr Ile Thr Ser Pro Gly Tyr Pro Gln Asp			
	35	40	45
Tyr Pro Ser His Gln Asn Cys Glu Trp Ile Val Tyr Ala Pro Glu Pro			
	50	55	60
Asn Gln Lys Ile Val Leu Asn Phe Asn Pro His Phe Glu Ile Glu Lys			
	65	70	75
			80

His 80	Asp 81	Cys 82	Lys 83	Tyr 84	Asp 85	Phe 86	Ile 87	Glu 88	Ile 89	Arg 90	Asp 91	Gly 92	Asp 93	Ser 94	Glu 95
Ser 100	Ala 101	Asp 102	Leu 103	Leu 104	Gly 105	Lys 106	His 107	Cys 108	Gly 109	Asn 110	Ile 111	Ala 112	Pro 113	Pro 114	Thr 115
Ile 120	Ile 121	Ser 122	Ser 123	Gly 124	Ser 125	Met 126	Leu 127	Tyr 128	Ile 129	Lys 130	Phe 131	Thr 132	Ser 133	Asp 134	Tyr 135
Ala 140	Arg 141	Gln 142	Gly 143	Ala 144	Gly 145	Phe 146	Ser 147	Leu 148	Arg 149	Tyr 150	Glu 151	Ile 152	Phe 153	Lys 154	Thr 155
Gly 160	Ser 161	Glu 162	Asp 163	Cys 164	Ser 165	Lys 166	Asn 167	Phe 168	Thr 169	Ser 170	Pro 171	Asn 172	Gly 173	Thr 174	Ile 175
Glu 180	Ser 181	Pro 182	Gly 183	Phe 184	Pro 185	Glu 186	Lys 187	Tyr 188	Pro 189	His 190	Asn 191	Leu 192	Asp 193	Cys 194	Thr 195
Phe 200	Thr 201	Ile 202	Leu 203	Ala 204	Lys 205	Pro 206	Lys 207	Met 208	Glu 209	Ile 210	Ile 211	Leu 212	Gln 213	Phe 214	Leu 215
Ile 220	Phe 221	Asp 222	Leu 223	Glu 224	His 225	Asp 226	Pro 227	Leu 228	Gln 229	Val 230	Gly 231	Glu 232	Gly 233	Asp 234	Cys 235
Lys 240	Tyr 241	Asp 242	Trp 243	Leu 244	Asp 245	Ile 246	Trp 247	Asp 248	Gly 249	Ile 250	Pro 251	His 252	Val 253	Gly 254	Pro 255
Leu 260	Ile 261	Gly 262	Lys 263	Tyr 264	Cys 265	Gly 266	Thr 267	Lys 268	Thr 269	Pro 270	Ser 271	Glu 272	Leu 273	Arg 274	Ser 275
Ser 280	Thr 281	Gly 282	Ile 283	Leu 284	Ser 285	Leu 286	Thr 287	Phe 288	His 289	Thr 290	Asp 291	Met 292	Ala 293	Val 294	Ala 295
Lys 300	Asp 301	Gly 302	Phe 303	Ser 304	Ala 305	Arg 306	Tyr 307	Tyr 308	Leu 309	Val 310	His 311	Gln 312	Glu 313	Pro 314	Leu 315
Glu 320	Asn 321	Phe 322	Gln 323	Cys 324	Asn 325	Val 326	Pro 327	Leu 328	Gly 329	Met 330	Glu 331	Ser 332	Gly 333	Arg 334	Ile 335
Ala 340	Asn 341	Glu 342	Gln 343	Ile 344	Ser 345	Ala 346	Ser 347	Ser 348	Thr 349	Tyr 350	Ser 351	Asp 352	Gly 353	Arg 354	Trp 355
Thr 360	Pro 361	Gln 362	Gln 363	Ser 364	Arg 365	Leu 366	His 367	Gly 368	Asp 369	Asp 370	Asn 371	Gly 372	Trp 373	Thr 374	Pro 375
Asn 380	Leu 381	Asp 382	Ser 383	Asn 384	Lys 385	Glu 386	Tyr 387	Leu 388	Gln 389	Val 390	Asp 391	Leu 392	Arg 393	Phe 394	Leu 395
Thr 400	Met 401	Leu 402	Thr 403	Ala 404	Ile 405	Ala 406	Thr 407	Gln 408	Gly 409	Ala 410	Ile 411	Ser 412	Arg 413	Glu 414	Thr 415
Gln 420	Asn 421	Gly 422	Tyr 423	Tyr 424	Val 425	Lys 426	Ser 427	Tyr 428	Lys 429	Leu 430	Glu 431	Val 432	Ser 433	Thr 434	Asn 435
Gly 440	Glu 441	Asp 442	Trp 443	Met 444	Val 445	Tyr 446	Arg 447	His 448	Gly 449	Lys 450	Asn 451	His 452	Lys 453	Val 454	Phe 455
Gln 460	Ala 461	Asn 462	Asn 463	Asp 464	Ala 465	Thr 466	Glu 467	Val 468	Val 469	Leu 470	Asn 471	Lys 472	Leu 473	His 474	Ala 475
Pro 480	Leu 481	Leu 482	Thr 483	Arg 484	Phe 485	Val 486	Arg 487	Ile 488	Arg 489	Pro 490	Gln 491	Thr 492	Trp 493	His 494	Ser 495
Gly 500	Ile 501	Ala 502	Leu 503	Arg 504	Leu 505	Glu 506	Leu 507	Phe 508	Gly 509	Cys 510	Arg 511	Val 512	Thr 513	Asp 514	Ala 515
Pro 520	Cys 521	Ser 522	Asn 523	Met 52											

-continued

500							505					510				
Thr	Ala	Val	Glu	Ala	Arg	Ala	Phe	Val	Arg	Lys	Phe	Lys	Val	Ser	Tyr	
		515					520					525				
Ser	Leu	Asn	Gly	Lys	Asp	Trp	Glu	Tyr	Ile	Gln	Asp	Pro	Arg	Thr	Gln	
	530					535					540					
Gln	Pro	Lys	Leu	Phe	Glu	Gly	Asn	Met	His	Tyr	Asp	Thr	Pro	Asp	Ile	
	545				550					555					560	
Arg	Arg	Phe	Asp	Pro	Ile	Pro	Ala	Gln	Tyr	Val	Arg	Val	Tyr	Pro	Glu	
				565					570					575		
Arg	Trp	Ser	Pro	Ala	Gly	Ile	Gly	Met	Arg	Leu	Glu	Val	Leu	Gly	Cys	
			580					585					590			
Asp	Trp	Thr	Asp	Ser	Lys	Pro	Thr	Val	Glu	Thr	Leu	Gly	Pro	Thr	Val	
		595					600					605				
Lys	Ser	Glu	Glu	Thr	Thr	Thr	Pro	Tyr	Pro	Thr	Glu	Glu	Glu	Ala	Thr	
	610					615					620					
Glu	Cys	Gly	Glu	Asn	Cys	Ser	Phe	Glu	Asp	Asp	Lys	Asp	Leu	Gln	Leu	
	625				630					635					640	
Pro	Ser	Gly	Phe	Asn	Cys	Asn	Phe	Asp	Phe	Leu	Glu	Glu	Pro	Cys	Gly	
				645					650					655		
Trp	Met	Tyr	Asp	His	Ala	Lys	Trp	Leu	Arg	Thr	Thr	Trp	Ala	Ser	Ser	
			660					665					670			
Ser	Ser	Pro	Asn	Asp	Arg	Thr	Phe	Pro	Asp	Asp	Arg	Asn	Phe	Leu	Arg	
		675					680					685				
Leu	Gln	Ser	Asp	Ser	Gln	Arg	Glu	Gly	Gln	Tyr	Ala	Arg	Leu	Ile	Ser	
	690					695					700					
Pro	Pro	Val	His	Leu	Pro	Arg	Ser	Pro	Val	Cys	Met	Glu	Phe	Gln	Tyr	
	705				710					715					720	
Gln	Ala	Thr	Gly	Gly	Arg	Gly	Val	Ala	Leu	Gln	Val	Val	Arg	Glu	Ala	
				725					730					735		
Ser	Gln	Glu	Ser	Lys	Leu	Leu	Trp	Val	Ile	Arg	Glu	Asp	Gln	Gly	Gly	
		740						745					750			
Glu	Trp	Lys	His	Gly	Arg	Ile	Ile	Leu	Pro	Ser	Tyr	Asp	Met	Glu	Tyr	
	755					760						765				
Gln	Ile	Val	Phe	Glu	Gly	Val	Ile	Gly	Lys	Gly	Arg	Ser	Gly	Glu	Ile	
	770				775						780					
Ala	Ile	Asp	Asp	Ile	Arg	Ile	Ser	Thr	Asp	Val	Pro	Leu	Glu	Asn	Cys	
	785				790					795					800	
Met	Glu	Pro	Ile	Ser	Ala	Phe	Ala	Gly	Glu	Asn	Phe	Lys	Val	Asp	Ile	
				805					810					815		
Pro	Glu	Ile	His	Glu	Arg	Glu	Gly	Tyr	Glu	Asp	Glu	Ile	Asp	Asp	Glu	
		820						825					830			
Tyr	Glu	Val	Asp	Trp	Ser	Asn	Ser	Ser	Ser	Ala	Thr	Ser	Gly	Ser	Gly	
	835					840						845				
Ala	Pro	Ser	Thr	Asp	Lys	Glu	Lys	Ser	Trp	Leu	Tyr	Thr	Leu	Asp	Pro	
	850				855						860					
Ile	Leu	Ile	Thr	Ile	Ile	Ala	Met	Ser	Ser	Leu	Gly	Val	Leu	Leu	Gly	
	865				870					875					880	
Ala	Thr	Cys	Ala	Gly	Leu	Leu	Leu	Tyr	Cys	Thr	Cys	Ser	Tyr	Ser	Gly	
				885					890					895		
Leu	Ser	Ser	Arg	Ser	Cys	Thr	Thr	Leu	Glu	Asn	Tyr	Asn	Phe	Glu	Leu	
			900					905					910			
Tyr	Asp	Gly	Leu	Lys	His	Lys	Val	Lys	Met	Asn	His	Gln	Lys	Cys	Cys	
	915						920					925				

-continued

Ser Glu Ala
930

<210> SEQ ID NO 70
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Ser Leu Thr Arg Lys Asp
1 5

<210> SEQ ID NO 71
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 71

Cys Asp Lys Pro Arg Arg
1 5

<210> SEQ ID NO 72
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 72

Ala Asn Ile Thr Val
1 5

<210> SEQ ID NO 73
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 73

Ala Asn Ile Thr Val Asn Ile Thr Val
1 5

What is claimed:

1. A human vascular endothelial growth factor A (VEGF-A) receptor antagonist comprising a substitution at an isoleucine residue corresponding to I83 in SEQ ID NO:4 or SEQ ID NO:13, wherein the substitution is a lysine, arginine or histidine, and the substitution results in a decrease in bioactivity as compared to wild-type VEGF.

2. The VEGF-A receptor antagonist of claim 1, wherein the VEGF-A receptor antagonist comprises an amino acid sequence of SEQ ID NO: 4, and I83 is substituted by a lysine, arginine or histidine.

3. The VEGF-A receptor antagonist of claim 1, wherein the VEGF-A receptor antagonist comprises an amino acid sequence of SEQ ID NO: 13, and I83 is substituted by a lysine, arginine or histidine.

4. The VEGF-A receptor antagonist of claim 1, which is expressed as at least one of subunits of a homodimer or heterodimer having two subunits.

5. The VEGF-A receptor antagonist of claim 1, wherein the antagonist contains one or more additional basic amino acid substitutions at the position(s) corresponding to the residues selected from the group consisting of positions E44, E67, E72, E73 and Q87 of SEQ ID NO: 4.

6. The VEGF-A receptor antagonist of claim 5, wherein the additional substitutions are selected from the group consisting of E72R and E73R of SEQ ID NO:4.

7. The VEGF-A receptor antagonist of claim 5, wherein the additional substitutions are selected from the group consisting of E72K and E73K of SEQ ID NO:4.

8. The VEGF-A receptor antagonist of claim 5, wherein the additional substitution is at a position corresponding to E44R or E44K of SEQ ID NO:4.

9. The VEGF-A receptor antagonist of claim 5, wherein the additional substitution is at a position corresponding to Q87K or Q87L of SEQ ID NO:4.

10. The VEGF-A receptor antagonist of claim 5, wherein the additional substitution corresponds to E67K of SEQ ID NO:4.

11. The VEGF-A receptor antagonist of claim 1, wherein interaction of the VEGF-A receptor antagonist and a native VEGF-A receptor results in inhibition of angiogenesis.

12. The VEGF-A receptor antagonist of claim 11, wherein the native VEGF-A receptor is kinase insert domain receptor (KDR).

13. The VEGF-A receptor antagonist of claim 5, wherein the antagonist contains the amino acid substitutions corresponding to E72R, E73R and I83K of SEQ ID NO:4.

14. The VEGF-A receptor antagonist of claim 5, wherein the antagonist contains the amino acid substitutions corresponding to E44R, E72R, E73R and I83K of SEQ ID NO:4.

135

15. The VEGF-A receptor antagonist of claim 1, further comprising an amino acid substitution at a position corresponding to C146 or C160 of SEQ ID NO:4.

16. The VEGF-A receptor antagonist of claim 1, wherein the amino acid substitution is at a position corresponding to C146S or C160S of SEQ ID NO:4.

17. The VEGF-A receptor antagonist of claim 1, further comprising an amino acid substitution at a position corresponding to A111 and/or A148 of SEQ ID NO: 4.

18. The VEGF-A receptor antagonist of claim 1, wherein the amino acid substitution is at a position corresponding to A111P and/or A148P of SEQ ID NO:4.

19. The VEGF-A receptor antagonist of claim 1, further comprising a toxin.

20. The VEGF-A receptor antagonist of claim 19, wherein the toxin is selected from the group consisting of a *Pseudomonas* exotoxin (PE), a Diphtheria toxin (DT), ricin toxin, abrin toxin, anthrax toxins, shiga toxin, botulism toxin, tetanus

136

toxin, cholera toxin, maitotoxin, palytoxin, ciguatoxin, texitilotoxin, batrachotoxin, alpha conotoxin, taipoxin, tetrodotoxin, alpha tityustoxin, saxitoxin, anatoxin, microcystin, aconitine, exfoliatin toxins A, exfoliatin B, an enterotoxin, toxic shock syndrome toxin (TSST-1), *Y. pestis* toxin and a gas gangrene toxin.

21. The VEGF-A receptor antagonist of claim 1, comprising a VEGF-A selected from the group consisting of VEGF₁₆₅ (SEQ ID NO:4), VEGF_{165b} (SEQ ID NO:13), VEGF₁₂₁ (SEQ ID NO:6), VEGF₁₄₅ (SEQ ID NO:8), VEGF₁₄₈ (SEQ ID NO:10), VEGF₁₈₃ (SEQ ID NO:15), VEGF₁₈₉ (SEQ ID NO:17), and VEGF₂₀₆ (SEQ ID NO:19), wherein I83 is substituted by lysine, arginine or histidine.

22. A pharmaceutical composition comprising the VEGF-A receptor antagonist of claim 1 and at least one excipient.

* * * * *